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An assessment of the sustainability of bioenergy production from algal feedstock

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In memory of my Grandfather, Professor Adam Jack Aitken and my Grandmother, Chandra Aitken

Declaration

I, Douglas Aitken, declare that this thesis and the work presented in it are my own and that it has been generated by me as the result of my own original research under the supervision of Dr Blanca Antizar-Ladislao. I confirm that: (i) This work was done wholly or mainly while in candidature for a research degree at this University; (ii) Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated; (iii) Where I have consulted the published work of others, this is always clearly attributed; (iv) Where I have quoted from the work of others, the source is always given; with the exception of such quotations, this thesis is entirely my own work; (v) I have acknowledged all main sources of help; and (vi) Where the thesis is based on work done by myself jointly with others I have made clear exactly what was done by others and what I have contributed myself.

Signed: ……

Date: ……

List of publications

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Abstract

Growing concerns regarding the impact of fossil fuel use upon the environment and the cost of production have led to a growth in the interest of obtaining energy from biomass. 1st and 2nd generation biomass types, however, are often criticised for their high energy requirements and environmental impacts. Algal biomass is considered a 3rd generation biomass which does not require arable land for cultivation, typically has a high productivity and can be converted to a wide variety of energy carriers. Despite research on the concept of producing energy from algal biomass dating back to the 1960s there has been limited commercial development and the environmental advantages are still in doubt. This thesis investigated the potential of algal biomass as a source of bioenergy feedstock by considering the cultivation and processing of localised species of algae and applying life cycle assessment (LCA) methodology to algal biofuel production systems. Experiments were conducted to examine the productivity of a wild algal species in wastewater and the potential recoverable bioenergy yields. The LCA studies drew together data from external studies, commercial databases, industrial reports and experimental work to assess the environmental impacts and the energy balance for each system considered.

The thesis investigated the generation of biofuel from both freshwater algal biomass and marine algal biomass. For both cases, the current state of research was examined and the gaps determined. Existing studies suggest the high intensity of microalgal biomass production (fertiliser requirements, high energy harvesting) greatly reduces the overall sustainability. Part of this thesis therefore investigated the possibility of a low input system of microalgal cultivation. A recommended approach was suggested using local species cultivated in wastewater as the nutrient source and a conversion strategy based on the characteristics of the dominant species.

The practicality and effectiveness of cultivating and processing locally grown algal biomass under low input conditions was determined by experiments that were conducted in the laboratory. Algal biomass was collected locally and cultivated in the laboratory using agricultural effluent as the nutrient source. The productivity of the algae was monitored alongside the uptake of nutrients. The effluent provided a good media for the cultivation of the wild algae and the nitrogen and phosphorous loading

of the effluent was reduced by as much as 98% for NH_4^+ and 90% for PO_4^{3-}. The algal biomass was also tested for its potential as a feedstock for bioethanol production as well as biochar alongside pyrolysis oils and gases. Compared to alternative biomass types tested, the algal biomass appeared to be a good candidate for bioethanol production providing a 38% recovery of bioethanol. The biomass appeared a less favourable substrate for energy recovery from pyrolysis but this process could be considered for carbon biofixation.

The sustainability of incorporating microalgal cultivation in wastewater treatment was tested by conducting a life cycle assessment of a large scale system. The life cycle assessment used Haifa wastewater treatment plant in Israel as a case study. The study compared algal cultivation with energy recovery to conventional nutrient removal (A_2O process) for enhanced nutrient removal within the wastewater treatment plant. It was found that the use of algal ponds for nutrient removal compared favourably to conventional treatment under specific conditions. These conditions were: the algal biomass is converted to both biodiesel and biogas and the algal biomass is converted to biodiesel, bioethanol and biogas. In these cases the energy balance was greater and the global warming potential and eutrophication potential were less. The conventional nutrient removal was, however, found to be the better method in terms of the acidification potential. Despite being the favourable method of nutrient removal the cultivation and processing of algae relies upon several key assumptions: high year round growth of algae, no contamination and access to a high land area for the cultivation ponds.

The sustainability of recovering bioenergy from the cultivation of macroalgae was also tested. A life cycle assessment was conducted investigating the energy return on investment and six environmental impacts for three cultivation methods and three process streams to convert the biomass to bioenergy. Cultivation and processing in Chile was used as a case study due to the depth of knowledge and availability of data. The cultivation scenarios were: bottom cultivation of *Gracilaria chilensis*, the long line cultivation of *Gracilaria chilensis* and the long line cultivation of *Macrocystis pyrifera*. The processing streams were: bioethanol, biogas and both bioethanol and biogas. Most of the data used in the life cycle assessment was

obtained from studies conducted in Chile and from communication with local fisherman. It was found that the bottom cultivation of *Gracilaria chilensis* and conversion to bioethanol and biogas produced the best energy return on investment (2.95) and was most beneficial in terms of the environmental impacts considered. Alternative circumstances were also considered which included new research (untested on a large scale) related to the value used for productivity and conversion of the biomass. This analysis indicated that an EROI of 10.3 could be achieved for the long-line cultivation of *Macrocystis pyrifera* and conversion to bioethanol and biogas alongside very limited environmental impacts. This result relies, however, upon favourable assumptions that have not yet been proven on a large scale.

The work conducted in this thesis highlights the potential of recovering energy from algal biomass. The experimental work and life cycle analysis of freshwater algal cultivation demonstrates the importance of using wastewater treatment as added value to the system. Maximising energy recovery by using a combination of conversion techniques was also shown to be key in providing the most sustainable solution. The sustainability of energy produced from macroalgae was established as being preferable to several conventional energy sources. Innovative methods to improve the system were also shown to greatly enhance the concept.

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Notation

LCA	Life cycle assessment
EU	European union
NREL	National renewable energy laboratory
NER	Net energy ratio
EROI	Energy return on investment
PBR	Photobioreactor
RP	Raceway pond
HRT	Hydraulic Retention Time
GWP	Global warming potential
Acid	Acidification
Eutro	Eutrophication
ODP	Ozone layer depletion
PO	Photochemical oxidation
HTP	Human toxicity potential
CO_2	Carbon dioxide
RUE	Radiation use efficiency
ISO	International organisation for standardization
PVC	Polyvinyl chloride

1 Introduction

Many global concerns are forcing us to rethink our current sources of energy. Some of the main issues are: climate change, fuel security and increasing and fluctuating costs [1]. Currently in a global context, fossil fuels make up the majority of our energy source with coal/peat, oil and natural gas accounting for 28.8%, 31.5% and 21.3% respectively [2]. Limiting the use of these energy sources is a key priority for most countries as a result of pledges to reduce greenhouse gas emissions [3]. Replacement sources of energy are necessary to replace the shortfall in fossil fuels, bio-energy is one such source [4]. The production of bio-energy is the result of converting organic matter into an energy carrier such as liquid fuel, gas, electricity and heat [5]. The organic matter can be sourced from many origins: dedicated energy crops, woodland residue municipal waste streams and industrial waste streams [5]. Targets to encourage the production of bio-energy have recently been implemented in many countries. The target for sustainable biofuel production for all European Union member states set by the European Commission is 10% of all transport fuels by 2020 [6]. Whilst hailed as a commendable aim when proposed, the target has led to the development of arable land for the production of 1st generation bio-energy crops (starchy crops) causing an increase in the cost of food crops [7]. Not only is there an impact on food prices, the true sustainability of biofuel produced from conventional 1st generation crops such as corn is in doubt [8]. Inefficient, expensive and environmentally damaging biofuel production cannot be considered a sustainable substitute for fossil fuels. Alternative 2nd generation (lingo-cellulosic crops) and 3rd generation (algal feedstock) substitutes are therefore being sought to replace fossil fuels [9]. Hydroponic cultivation of biomass has been receiving attention as a potential solution as non-arable land can be used [10-13].

The purpose of this thesis is to investigate the potential for development and implementation of sustainable bioenergy from algal biomass. The research conducted uses life cycle assessment (LCA) methodology and laboratory experimentation to understand the potential of different systems to produce bioenergy from algal biomass. The current research conducted on this area is considered first with discussion regarding the challenges facing the development. Experimental work is

then included which investigated the potential for cultivation of the biomass and subsequent conversion to energy. This is followed by several case studies where LCA methodology is used to determine the sustainability of developing systems to cultivate algal biomass and recover energy.

1.1 Development of bioenergy production from algal biomass

1.1.1 A background to bioenergy from algae

Research conducted on the subject of recovering energy from algal biomass has always been most prevalent around times of energy insecurity [14]. This is the case for both freshwater algae and marine algae [15]. Research investigating the potential of energy recovery from freshwater algae was first initiated in the 1950s [16]. The first concept was derived as a result of ideas for the use of algal biomass in wastewater treatment ponds where the biomass was used as an oxygen source for oxidising bacteria [16]. The first published research investigating the anaerobic digestion of algal biomass from wastewater stabilisation ponds was published in 1957 [17]. Studies conducted investigating the energy recovery possibilities of aquatic biomass became more prominent in the late 1970s as a result of the oil crisis during this decade. Research continued to mainly focus upon the production of methane [18] although attention started to spread to the production of biodiesel. The US Department of Energy funded the Aquatic Species Program [14], the main consideration of which was the recovery of biodiesel from microalgae. The program ran for 20 years and despite positive results being obtained was gradually phased out as the US recovered from the energy crisis.

The conversion of macroalgae (seaweed) to bioenergy has followed a similar path to microalgae albiet receiving less attention as the cultivation of macroalgae is less flexible and the variety of potential energy products are limited in comparison to microalgae [19]. The Marine Biomass program was initiated in the 1970s to investigate the potential for cultivating macroalgae and converting the biomass to biogas. Similarly to microalgal research, towards the end of the 1970s there was a decline in interest related to this area due to a return to fuel security and reduced funding.

Due to fears related to climate change and fuel security there has been a resurgence in research interest into the conversion of algal biomass to bio-energy [20-22]. The current research is broad, considering many different species of both micro and macro algae as well as a large variety of bio-energy products.

1.1.2 Current research initiatives

Current research on algal bioenergy is varied however the main focus has been on microalgal biomass with conversion to biodiesel. Figure 1-1 displays the number of articles published by search term topic from 2003 to 2013 on ScienceDirect. The value for the year 2013 was taken on 4th April 2014.

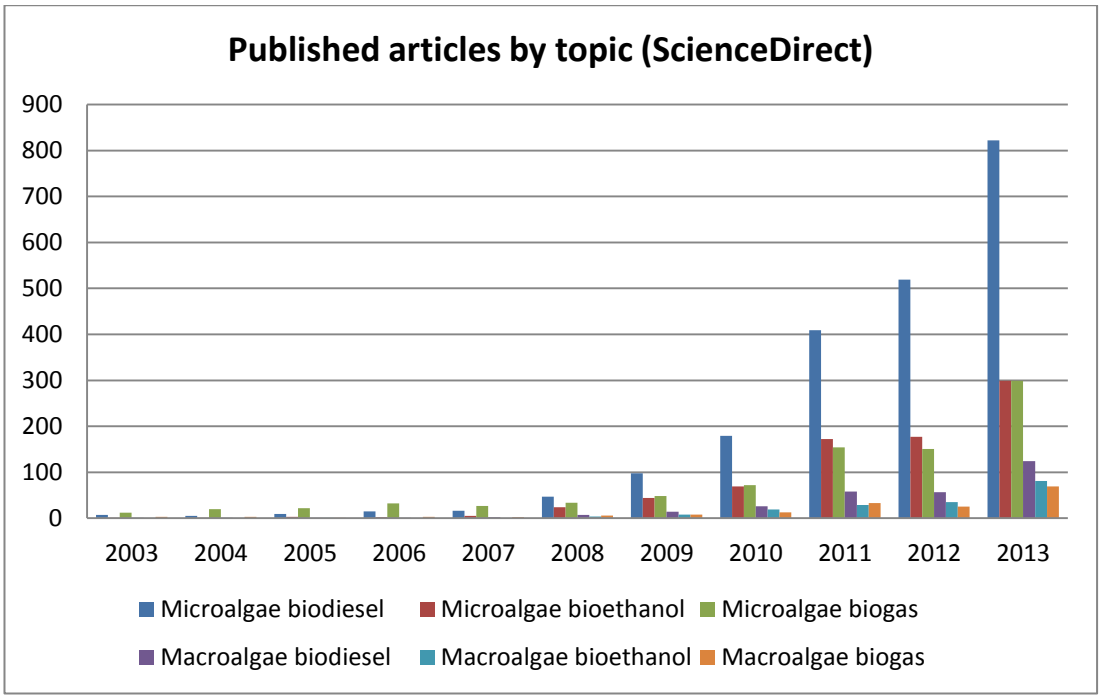


Figure 1-1 Search results for each search term from ScienceDirect

It is clear from Figure 1.1 that research conducted investigating bio-energy from both microalgae and macroalgae has increased rapidly over the past 10 years, particularly for microalgae and biodiesel production. It can be observed that previous research had focussed on biogas generation but this was overtaken by biodiesel generation around 2008 and more recently slightly by bioethanol production. The reason for the interest in biodiesel production is partly because biodiesel is considered a more valuable fuel than biogas as it can be used as a direct substitute for diesel [23], and

because species of algae were found to contain high amounts of lipids (fats and oils) that can be easily converted to methyl esters (biodiesel) [24]. Indeed, some species of algae are reported to contain up to 70% lipids [25]. The extraction of lipids from biomass is well understood and thus the implementation of algal biomass into a system similar to that for using soy bean can potentially be considered fairly simple [26]. Nevertheless the commercial viability of producing bioenergy from algal biomass has been in doubt due to the numerous complexities of the full life-cycle [27]. Industry and researchers have been attempting to increase bioenergy yields through strain selection, stressing biomass (e.g. low nitrogen conditions) and increasing biomass productivity using methods such as CO_2 injection and extensive artificial lighting [28-31]. Nevertheless these changes often lead to increased energy consumption and adverse environmental impacts [27, 32]. Recent research has concentrated on considering the full life cycle of algal bioenergy and pinpointing areas which are reducing the viability of commercial implementation [10, 33, 34]. Several studies have demonstrated that producing only biodiesel from algal biomass is not sufficient to make the process sustainable or viable [35, 36]. Both studies suggest that anaerobic digestion of the residual waste from the esterification process (conversion of oils to methyl esters) is necessary for the greatest recovery of energy from the biomass. The understanding of the viability of various processing scenarios is key to successful commercial implementation of converting algal biomass to bioenergy. For this reason at this stage of research the use of life cycle analysis is necessary to test specific scenarios using data which has been and continues to be generated.

1.1.3 Life cycle assessment in algal bioenergy

Recent LCA studies have developed from being relatively general to focussing more on specific scenarios where improvements have been made over previous studies [27, 33]. The majority of studies have focussed upon the use of microalgal biomass [27, 32, 33, 35] with limited interest in macroalgal biomass. Some of the first LCA studies acknowledged the potential for algal bioenergy but the results suggested the technology was unable to compete with conventional biofuels in terms of the energy balance and environmental impacts [27, 35]. As the concept of converting algal

biomass to energy is relatively recent, there is still a great amount of scope to improve system processes to enhance the overall sustainability. Jorquera *et al.* [37]. investigated which method of cultivation was most effective in terms of the net energy balance where it was found that raceway ponds were greatly superior to photo-bioreactors (twice the energy ratio). As mentioned, several studies have considered the production of both biodiesel and biogas [10, 35]. To reduce the environmental impacts and energy intensity of the cultivation and processing stages, some studies have considered the potential co-benefits of alternative cultivation methods such as using wastewater as a growth medium or injection of flue gas to increase productivity through CO_2 uptake whilst providing a form of carbon sequestration [33, 38, 39].

The study of bioethanol production through LCA is limited in comparison to biodiesel and biogas production, yet some studies have been conducted for both microalgae and macroalgae. In the case of microalgae, research has been conducted investigating the potential for intracellular production of bioethanol via cyanobacteria with promising results with respect to the energy balance [40], yet this research remains at a very early stage. With regards to macroalgae, Alvarado-Morales *et al.* [41] conducted an LCA study comparing biogas production to combined bioethanol and biogas in Nordic conditions with the suggestion that both produced a positive energy ratio (>1) with just biogas being preferable in terms of its energy ratio. Langlois *et al.* [42] considered the generation of biomethane from seaweed with comparison to natural gas, the biomethane being inferior in terms of environmental impacts given current methods to produce the seaweed. An earlier study by Aresta *et al.* [43] investigated gasification of macroalgae suggesting the process compared favourably to gasification of microalgae.

When considering bio-energy generation, LCAs generally consider the energy generation potential, the energy consumption and the environmental impacts of each of the unit processes as well as the system as a whole. This allows different process streams to be compared to alternative methods for energy recovery and alternative inputs to be tested. These results are generally scaled to one unit which is called the functional unit. When bio-energy is concerned this unit is often a measure of energy,

for example one mega joule (1 MJ), one kilowatt-hour (1 kWh) or for biofuels it can be vehicular distance travelled. A common method to test the energetic sustainability is to calculate the energy balance or energy ratio. The energy balance is usually the total energy produced minus the energy required to produce that energy [35], naturally a value above zero is necessary for the energy to be deemed to have a net positive energy balance which is desirable. In contrast, the energy ratio is the total energy produced divided by the total energy required to produce that energy, in this instance a value above 1 is necessary for a net positive energy ratio although Hall *et al.* [44] determined that a value of at least 3 is necessary for an energy production system to be considered sustainable. This is the value determined to account for the energy required to extract, refine and transport the energy carrier [44] Table 1-1 displays the energy ratio values for many of the recently published life cycle assessment studies investigating the production and conversion of microalgal biomass to energy carriers. There is currently insufficient LCA studies conducted for energy recovery from macroalgae to produce a similar table.

The reported values of energy ratios differ greatly between studies and the conditions considered within studies. Raceway ponds were the method of cultivation chosen by the majority of the LCA studies due to the lower material and energy requirements. Where PBRs have been examined the bioenergy carrier produced was calculated to have a lower energy ratio than ponds [31, 36]. The highest net energy ratio recorded was by Stephenson et al with a value of 5.82 providing an energy saving of 85% over fossil derived diesel. In comparison Clarens *et al.* [10] reported the energy consumption of producing 317 GJ of switchgrass energy as 29 GJ, a net energy ratio of 10.9. In many cases the NER is reported as being just above 1 or slightly below depending upon the conditions [10, 35] suggesting the energetic sustainability is certainly not definite for algal bioenergy. Where the NER values are high, often particular conditions are assumed, low nitrogen in the feedwater, wet biomass processing, CO_2 is pumped into the pond or wastewater supplies nutrients [10]. Although these conditions should be beneficial to the system, the practicality of their implementation has not yet been given much study.

Table 1-1 Energy ratio values of recent LCA studies of microalgae to bioenergy

Study	Method	Bio-energy product	Conditions	NER
1	RP	Biodiesel and biogas	Sufficient nutrients/Dry processing	0.98
2	RP	Biodiesel and biogas	Sufficient nutrients/Wet processing	3.55
3	RP	Biodiesel and biogas	Low nitrogen/Dry processing	1.25
4	RP	Biodiesel and biogas	Low nitrogen/Wet processing	4.34
5	RP	Algae		1.06
6	RP	Biogas to bioelectricity	Virgin CO_2 Carbon capture Flue gas Wastewater supplementation	1.06 1.14 1.69 1.72
7	RP	Biodiesel and biogas to bioelectricity	Virgin CO_2 Carbon capture Flue gas Wastewater supplementation	0.65 0.72 1.11 1.13
8	RP	Biodiesel and direct combustion to bioelectricity	Virgin CO_2 Carbon capture Flue gas Wastewater supplementation	0.99 1.36 1.99 1.99
9	RP	Direct combustion to bioelectricity	Virgin CO_2 Carbon capture Flue gas Wastewater supplementation	1.53 2.90 4.10 4.09
10	RP	Oil	-	3.05
11	PBR	Oil	-	1.65
12	RP	Biodiesel	-	5.82
13	PBR	Biodiesel	-	0.19
14	RP	Biodiesel	Centrifuge dewatering	1.63
15	RP	Biodiesel	Filter press dewatering	3.03

(Note: 1-4: [35], 5-9: [10], 10-11:[37], 12-13:[32], 14-15: [45])

Reported environmental impacts also vary greatly between studies particularly as studies often use different impact categories and determination methods. The most common environmental impact is global warming potential (GWP) or simply CO_2 emissions. In most studies the global warming potential is positive, meaning there is a net positive emission of CO_2 [27, 32, 35] although some report a negative emission of CO_2 [45] suggesting an uptake of CO_2 as a result of the cultivation stage. CO_2 uptake is common for some biofuel types [27] however the majority of studies suggest it would not be the case for algal bioenergy due to the high emissions resulting from material (concrete, steel, fertiliser) production as well as direct emissions from energy generation (electricity and heat) [27]. For other environmental

impact categories, the results of scenarios investigating bioenergy from algal biomass suggest that in some areas, eutrophication and land use compare favourably to alternative bioenergy crops [27, 35]. Other impacts, water use, ionizing radiation, photochemical oxidation, marine toxicity however do not compare well [27, 35]. The results again depend very much upon the assumed conditions and the impact categories considered.

The study by Langlois *et al.* [42], the authors investigate the environmental impacts of producing biomethane from marine macroalgae using a base case and an 'ecodesigned' case. When comparing these cases to natural gas the reference case was almost always the worst for each impact category with the 'ecodesigned' case occasionally being preferable, for example for ozone layer depletion and fossil depletion. The macroalgal processes provide the most favourable results for marine and freshwater eutrophication as macroalgae cultivation is considered to provide some contamination removal in water.

There is no strong correlation between most LCA studies that have been conducted to date, some show the potential of algal biomass to bioenergy to be great although most suggest that improvements are necessary before a system can compare favourably to conventional biofuels or energy carriers from fossil fuel. There is also a lack of studies considering macroalgae as a source of biomass and the production of bioethanol from both micro and macroalgae.

1.2 Hypothesis, overall aims and objectives

There is still much research necessary to understand whether the cultivation and processing of algal biomass has the potential to provide a significant amount of sustainable bio-energy. The scenarios in which systems can be implemented to develop algal biomass and convert it to energy are extremely varied and therefore is unlikely to be one single best solution to producing energy from algal biomass. The most sustainable solutions are likely to be those which are designed considering particular cases depending upon local conditions and demand. To provide an advantage over other energy and bioenergy types it is also likely that other benefits will be required alongside the production of bioenergy from algal biomass.

The hypothesis of this thesis is that the production of bioenergy from algal biomass can be sustainable. This thesis will consider the current understanding of algal biofuel production and assess the sustainability of implementing different systems in specific scenarios with the use of existing data and data produced in this study. The aim of the thesis is to present the best scenario for recovering energy from algal biomass.

The objectives of this thesis are as follows:

1. Review current state of knowledge
2. Investigate the growth of indigenous species in wastewaters using open systems
3. Investigate the conversion of algal biomass using biological and thermal processes
4. Investigate the sustainability of incorporating algal cultivation and conversion to energy into the wastewater treatment process as a method of nutrient removal and compare this with conventional alternatives
5. Investigate the sustainability of macroalgal cultivation and processing to bioenergy
6. Compare the sustainability of the different cultivation and conversion strategies and identify what further research is required

1.3 Outline of the thesis

The thesis is designed to first consider the current state of research of algal bioenergy and investigate the limitations that are impeding the commercialisation of the concept. Chapter 3 presents experimental work conducted to assess the practicality of low impact freshwater algal biomass cultivation and subsequent processing to different bioenergy carriers. Chapter 4 investigates the sustainability of a system to cultivate algal biomass in wastewater treatment plant effluent to reduce nutrient concentrations. This chapter uses life cycle assessment methodology to compare a conventional nutrient removal process to the algal cultivation system for a case study in Israel. The study considers the energy balance and several environmental impacts. Chapter 5 investigates the sustainability of macroalgae cultivation using life cycle

assessment methodology with cultivation in Chile as a case study. The results of the thesis are discussed in Chapter 6 and conclusions and recommendations are made in Chapter 7.

2 Limitations and focus points of bioenergy from algal biomass

2.1 Introduction

Research investigating the potential for bio-energy recovery has seen a strong resurgence from the late 2000s due to climate change and fossil fuel security and costs [46]. Nevertheless the concept of converting algal biomass to bioenergy remains at an early stage with many obstacles needing to be overcome [27, 35]. Many of the issues regarding bio-energy from algal biomass, particularly microalgae, were encountered early in the development of algal bio-energy. Some of the issues encountered were strain selection and contamination, inconsistent productivity and energy intensive harvesting methods [14, 47, 48]. These barriers remain, prohibiting the commercial viability of algal bio-energy. Some solutions have been developed to overcome these obstacles such as the use of fertilisers for enhanced growth, artificial lighting and closed reactors for reducing contamination. Nevertheless these methods often reduce the overall sustainability of the energy recovery due to higher energy costs and a greater environmental footprint [32].

The aim for the generation of bio-energy is to produce a sustainable energy source. There is no definite description of this but in general it denotes a source that produces a positive energy balance (or net energy ratio above one) with limited environmental impacts or impacts that are at least lower than those of conventional fossil fuels. There is currently no specific criteria for sustainable bio-energy however ISO standards are being developed as ISO PC 248 [49]. The current criteria are very broad and differ between countries and bioenergy types. In Europe the main focus is on biofuels due to the 2009/28/EC Directive requiring EU member states to incorporate 10% biofuels into their transport fuel use by 2020 [50]. In the UK under the government's Renewable Transport Fuel Obligation all biofuel suppliers must demonstrate the carbon savings of their fuel and report the sustainability considering:

- Carbon conservation
- Biodiversity conservation
- Soil conservation
- Sustainable water use
- Air quality
- Biomass production does not adversely affect workers' rights and working relationships
- Biomass production does not adversely affect existing land rights and community relationships

The generation of bio-energy from algae must comply with these criteria. In general, studies are looking at where the concept can reduce carbon dioxide emissions by lowering energetic and material inputs as well as many other environmental impacts. This chapter will look at the current research and which areas are prohibiting algal biofuels reaching the desired criteria in terms of their sustainability and what options are possible for improvement.

2.2 Biomass cultivation and species selection

Biomass cultivation is one of the most intensive processes in the production system of any bio-energy generation system [27]. For algal cultivation, the impact of the cultivation step depends very much upon the species of algae and the conditions of cultivation. The variation in cultivation methods between microalgae and macroalgae are great, and therefore these two types of algae are considered separately.

2.2.1 Microalgae

As mentioned in the introductory chapter the two main methods of cultivating microalgae are through the use of raceway ponds and photo-bioreactors [30]. Both methods have advantages and disadvantages and their use depends upon the final product sought and the operating conditions. Table 2-1 provides a general overview of the advantages and disadvantages associated with both methods of microalgal biomass cultivation.

Table 2-1 The advantages and disadvantages of two different common methods for algal biomass cultivation

	Advantages	Disadvantages
Raceway ponds	• Low operating energy consumption and costs • Relatively low capital costs and embodied energy • Well researched	• Low productivity rates • Dilute biomass • Poor contamination control • Large land area required • High water loss
Photo-bioreactors	• High productivity rates • Concentrated biomass • Conditions easily controlled • Small land area required	• High operating energy consumption and costs • High capital costs and embodied energy

The table indicates the general understanding of both methods at the current time, ponds are more widespread for high volume production of microalgal biomass due to the relatively lower capital and operating costs [37] and photo-bioreactors currently provide a means for researching specific algal strains and controlling conditions [37].

2.2.1.1 Raceway ponds

The concept of using ponds for the cultivation of algal biomass was first published by Oswald *et al.* in 1955 [51]. Ponds were an existing feature of wastewater treatment plants where the concept of using algal biomass for oxygen production came about [51]. The idea to use ponds as the cultivation method was continued by these researchers, Oswald and Golueke [52], who suggested using 40 ha ponds to cultivate algal biomass for conversion to methane to use as an energy source. During the 1970s and 1980s several research groups investigated the use of large scale raceway ponds for the generation of algal biomass to methane with mixed results [14, 47, 53]. The researchers behind the aquatic biomass program cultivated microalgal biomass in ponds of up to 0.25 ha with most of the early research being conducted in test programs conducted in California and Hawaii [14]. The concept was further developed in New Mexico where 1,000 m^2 ponds were constructed and operated. The ponds incorporated the use of CO_2 injection to enhance productivity rates with promising results. The program reported relatively reasonable species

control and a maximum growth rate of 50 g/m^2/day. Growth rates however were not consistent and were reduced by low temperatures. The program reported that the best method of algal cultivation was to allow a natural species to develop. The pond was first inoculated with *C. Cryptica CYCLO1* and provided productivities of 30 g/m^2/day in August but reduced to half in September and October [14]. The species, *Micractinium Minutum* was used after this period as it is considered a more suitable species for colder temperatures. The biomass however only managed a productivity of around 10 g/m^2/day in November and 3.5 g/m^2/day in December. The researchers also considered the carbon sequestration potential and estimated that CO_2 utilisation from flue gas injection was generally very high at around 90% with the remaining 10% off-gassing to the environment. Of the two ponds constructed one was lined with plastics and the other unlined. The ponds were inoculated with the alga *Tetraselmis suecia* and the productivities in each pond were similar but relatively low at 11 and 10 g/m^2/day for the lined and unlined pond respectively. The ponds were mixed with paddle-wheels, the power consumption of which was generally less than 0.1 W/m^2. The unlined pond was recorded to have a power consumption of approximately 0.04 W/m^2 , the equivalent of a 40 W light bulb for the 1,000 m^2 pond [14].

Similar work was conducted in Israel by Professor Gedaliah Shelef in the 1970s and 1980s who oversaw the construction and operation of two 1,000 m^2 ponds [53]. The purpose of the ponds was to investigate the potential for using algal biomass for oxygen production in high rate ponds designed for wastewater treatment, similar to concept developed by Oswald in the 1950s [51]. The final product was assumed to be used for animal feed instead of a bio-energy feedstock. Natural species were allowed to dominate and the dominance was recorded. The main species recorded were *Scenedesmus* sp., *Micractinium* sp., *Chlorella* sp. and *Euglena* sp. *Euglena* sp. and *Scenedesmus* sp. were dominant in winter and *Micractinium* sp. and *Chlorella* sp. were dominant in summer. The productivity of the algae in one of the large ponds was recorded using a direct count of algal cells from November to July in 1976/1977, the greatest recorded productivity was in July with a productivity of 50 g/m^2/day and the lowest in December, 0.4 g/m^2/day. The work conducted by Professor Shelef found that algal species dominance was not controllable, productivity rates varied

greatly between seasons but sewage provided a 'complete medium' for the algae and growth was not nutrient limited. These findings largely support those found in the USA during the aquatic species program [14].

From this early research it can be assumed that ponds can work effectively at sizes of around 1,000 m^2, generally in a raceway formation (long length with short width) with the water kept in motion using either paddlewheels or a method of aeration. Ponds were built using concrete and either lined with PVC or crushed stone. The use of a CO_2 sump has been reported as being effective in minimising carbon limitation [14].

In the LCA study by Jorquera *et al.* [37] the authors used a raceway pond for algal cultivation is 100 m by 10m with a depth of 35 cm constructed using concrete with a 2 mm PVC lining. The energy consumption through private communication with early developers of the concept was assumed to be 3.72 W/m^3 or 1.3 W/m^2 given the depth of pond, a fairly similar value to that recorded by the aquatic species program (1 W/m^2). The biomass productivity was based on *Nannochloropsis* sp. and was assumed to be 0.035 g/l/day which given a depth of 35 cm equates to an areal productivity of 11 g/m^2/day. This assumption is fairly conservative given the reported yields of Shelef [47] and the aquatic biomass program although it is likely higher than most winter rates. The study by Lardon *et al.* [35] assumed a very similar set-up with a slightly lower depth of 30 cm. However the productivity under normal (sufficient) nitrogen conditions was assumed to be 24.75 g/m^2/day, a value far higher than that of Jorquera *et al.*[37]. The inputs of this study were based around the use of the species *Chlorella* sp. Pressurised CO_2 was also assumed to be an addition to the set-up with the assumption that the injection of CO_2 required 22.2 Wh/kg CO_2 taken from the work conducted by Kadam [48]. Another study with a slightly different approach to estimating the productivity of algae cultivated in ponds is that of Clarens *et al.* [27] where the authors use data from three pilot scale plants in Brawley, California [54], Roswell, New Mexico [48] and San Juan, New Mexico [55] to calculate the radiation use efficiency (RUE) from the climate data of the area. They were able to calculate a mean RUE and use that value to calculate values of productivity for three hypothetical geographical locations for development of

raceway ponds: California, Virginia and Iowa. The productivities calculated for each given the climatic data used was 12.9, 11.0 and 9.45 g/m^2/day respectively. Clarens *et al.* [27] based their calculation of paddlewheel energy consumption on paddle wheel aeration research [56] finding a most likely value of 0.37 W/m^2 of pond area.

Research conducted examining the use of raceway ponds for algal cultivation to date suggests large concrete, lined or unlined ponds can be an effective method of producing algal biomass. A length of about 100 m, a width of 10 m and a depth of between 25 cm and 50 cm could be considered useable. Productivities depend very much upon the location of the pond as the productivity depends upon temperature, available radiation and species dominance [34]. Additionally, due to seasonal variations in these conditions the productivity varies drastically between winter and summer. Even in locations that are deemed highly suitable for biomass growth, the productivity can vary from almost 0 in winter up to about 50 g/m^2/day making a reasonable estimate of productivity very difficult. As suggested by most studies the control of species dominance in ponds is virtually impossible which can have a great impact upon the value of the biomass cultivated. Table 2-2 below displays the general ideal characteristics of raceway ponds.

Table 2-2 Example of general raceway pond characteristics

Characteristic	Value	Key references
Dimensions	100 by 10 by 0.25-0.5 m	[14, 35, 37]
Material	Concrete cavity/PVC lining	[14, 37]
Productivity	0.4-50 g/m^2/day	[14, 27]
Power	0.37-1.3 W/m^2	[14, 27, 37]

2.2.1.2 Photo-bioreactors

The main alternative method to raceway cultivation is the use of photo-bioreactors, enclosed plastic or glass tubes containing the algal biomass and growth medium. The use of photo-bioreactors however is mainly restricted to laboratories and pilot scale plants in their current state [37]. Photo-bioreactors have the benefit of being able to produce algae with greater productivity rates and control than ponds. Light can be used more efficiently by reaching more of the algal cells, air/CO_2 injection allows a greater mixing of CO_2 with the water, temperatures are easily controlled by heating

or cooling the tubing and contamination by other organisms is greatly reduced by using a sealed system [57]. These characteristics make the use of photo-bioreactors ideal for laboratory studies and experimenting with new species and techniques. Their use for large scale production of microalgae however is likely to be limited due to the energetic intensity of production and operation as well as the higher economic costs [37].

Large scale PBRs are generally constructed of thin tubing or panels produced from plastics. Molina *et al.* [58] suggest that tubular PBRs are the most effective design with airlift devices to pump air through the culture. This is contradicted however by Jorquera *et al.* [37] who found that flat-plate bioreactors provided a better Net Energy Ratio (NER) over tubular PBRs in their energy balance study. Molina *et al.*. [58] designed a tubular photo-bioreactor for outdoor pilot-scale production of the microalgae *Phaeodactylum tricornutum* using plexi-glass material (Fig 2-1).

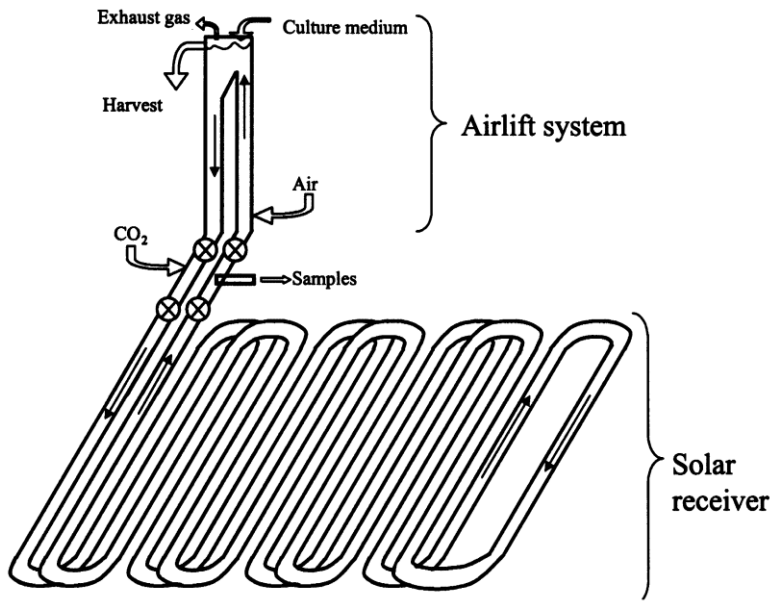


Figure 2-1 Example diagram of a photobioreactor
(Note: taken from Molina-Grima *et al.* [58])

Using this set-up a maximum biomass productivity of 1.9 g/L/day was obtained from outdoor cultivation which reduced to 1.2 g/L/day in the spring cultivation period. Both of these figures are potentially extremely high compared to pond cultivation

providing at least 20 L of medium can be supported by 1 m^2. Despite high productivity rates the sustainability of using such methods on a large scale are in doubt due to the materials and energy used. Jorquera *et al.* [37] calculated a net energy ratio of 0.07 and 1.65 for oil produced from biomass cultivated using tubular and flat plated PBRs respectively. In the same study, a NER of 3.05 was calculated for the use of raceway ponds. Similarly, Stephenson *et al.* [32] found the fossil energy requirement of biodiesel produced from raceway cultivated algal biomass (*C. vulgaris*) was an order of magnitude less than that required for the biodiesel produced from airlift photo-bioreactor produced biomass. The global warming potential was also considerably less due to lower energy and resource consumption. Table 2-3 displays typical characteristics and inputs for PBR systems.

Table 2-3 Example of general photobioreactor characteristics

Characteristic	**Value**	**Key references**
Dimensions	Diameter 0.06 m Length 80 m	[58]
Material	Plexi-glass, perspex	[58] [32]
Productivity	1.2-1.9 g/L/day	[58]
Power	2500 W/m^3 (tubular) 53 W/m^3 (flat plate)	[37]

2.2.2 Macroalgae

The method of cultivating macroalgae (saltwater algae) depends entirely upon the species of macroalgae and how that algae reproduces and develops. Some species of macroalgae such as *Ulva sp.* can be cultivated in a similar way to microalgae using tanks and ponds as they are free-floating [15] however most require a substrate to which they can attach. These types of algae which require a substrate can be planted in the seabed (Bottom planting) or if suitable they can be grown on ropes or a similar structure (Long-line cultivation) [59-61]. Table 2-4 displays the advantages and dis-advantages of each of the cultivation methods.

Table 2-4 Advantages and disadvantages of different macroalgae cultivation methods

Method	Advantages	Dis-advantages
Bottom planting	Well understood Relatively productive Low material input	Area limited Highly labour intensive Biomass loss
Long-line cultivation	High productivity Large area potential	High material and energy requirement (nursery grow out and offshore placing/harvesting) High labour requirement
Tank/Pond cultivation	High productivity	Very high material and energy requirement

2.2.2.1 Bottom planting

Bottom planting is a close to shore technique used to cultivate species of macroalgae that require a substrate with which to attach themselves to. The macroalgae thalli (vegetative tissue) can either be planted directly into the sea floor alternatively tied to rocks or weighted tubing to give a stronger substrate [60]. The planting process requires a high level of labour input using fishing vessels and divers. However in some cases planting only needs to be conducted once every two or more years whilst the biomass can be harvested several times a year [60] (Personal communication with local seaweed farmers in the south of Chile, March 2013).

Like all biomass, the productivity rates of the biomass depend upon the location of the cultivation area. Much research has been conducted in Chile investigating the cultivation of a species of macroalgae called *Gracilaria chilensis* which is used for the production of agar [61] and is considered one of the only profitable species to be cultivated there [61]. *G. chilensis* can be cultivated either sub-tidally or inter-tidally. Sub-tidal systems tend to be more productive: typical estimates of productivity are from 91-149 t (w.w.)/ha/yr whereas the productivity of inter-tidally cultivated *G. chilensis* was estimated to be less than 72 t (w.w.)/ha/yr [60] Despite the higher biomass productivity for sub-tidal cultivation systems, the intensity of cultivation is also higher requiring divers for both planting and harvesting. Inter-tidal systems can be planted without the use of divers and boats.

2.2.2.2 Long-line cultivation

A more common method of macroalgal cultivation is the use of long-lines. The long-line cultivation method uses ropes to which macroalgal spores or thalli are attached to and are subsequently placed offshore. The spore inoculation method uses the transfer of spores from fertile thalli to thin cultivation ropes to which they become attached. In a tank they are then allowed to develop in ideal conditions using artificial lighting, air diffusion and a suitable temperature [62]. Following a period of around 60 days, the culture ropes can be wound around larger structural ropes and deployed off-shore in large cultivation areas [62]. Figure 2-2 depicts a typical off-shore culture installation of 1 hectare using concrete blocks and buoys to restrict movement of the ropes.

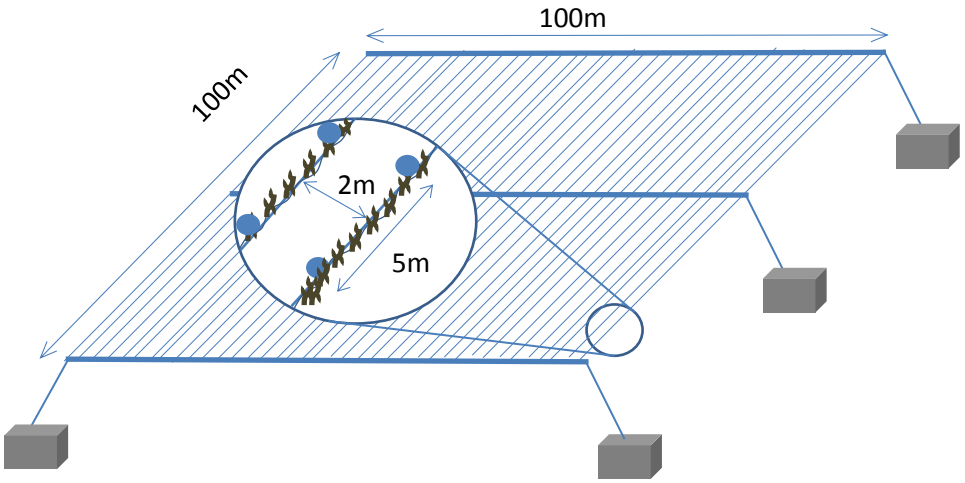


Figure 2-2 Typical cultivation set-up for 1 hectare of long-line macroalgae cultivation

Depending upon the location ,the cultivation period can last for around 6 to 9 months before being harvested. This method of cultivation can apply to many types of macroalgae although the focus has been mainly upon brown algae (*Macrocystis pyrifera*, *Laminaria digitata*, *Saccharina japonica*) [32, 62-64]. Very high values have been recorded for this method of macroalgal cultivation, similarly to bottom cultivation much of the research has been conducted in Chile. The main species considered in Chile has been *M. pyrifera* and productivity values of 22 to 25 kg (w.w.)/m/year have been obtained for the spore inoculation method [62]. Much

greater values have however been recorded for a slightly different cultivation method where selected gametophytes (fertile thalli) were developed and attached to the ropes. Using this technique, productivity values of up to 80 kg/m/year have been recorded. This method of biomass cultivation offers extremely high productivity compared to bottom culture although the inputs are also high. The spore inoculation and development requires the use of a hatchery with a number of inputs (temperature control, lighting, fertilisers etc.) [62]. The offshore cultivation also requires a high material input due to rope and concrete use as well as large vessels for deployment.

An alternative technique for long-line cultivation is the tying of biomass thalli to the culture ropes. This has proved to provide relatively high biomass productivity although the labour costs of tying the thalli are likely to be high as well [65] and an existing source of biomass is necessary. Productivity rates of 1.7 to 2.8 kg/m/month (7.6 to 12.5 t/ha/yr assuming 2 metres distance between lines) have been recorded for this method of cultivation using *G. chilensis* in the south of Chile [65, 66].

2.3 Carbon mitigation

2.3.1 Microalgae

Although not all algal types are photosynthetic, the majority of the more common species tend to be and therefore uptake carbon dioxide (CO_2) as they develop [67]. The atmospheric CO_2 concentration is currently at 0.0398% [68] which is able to support photosynthetic growth, the cultivation and use of algal biomass can therefore provide a source of CO_2 removal by using atmospheric CO_2. It is however possible that growth can be increased as a result of supplying a more concentrated source of CO_2 [69]. During the aquatic species program the use of CO_2 was investigated for its potential to increase productivity rates and utilisation efficiency. In the work undertaken at the New Mexico pilot plant, CO_2 was injected into carbonation sumps at a depth of between 0.6 and 0.9 m with a utiliasation efficiency estimated to be 90% [14].

The ideal scenario for algal cultivation is to use flue gas as a source of CO_2 thereby avoiding emissions that would otherwise enter the atmosphere. In 2002 research was conducted by the National Renewable Energy Laboratory (NREL) and the US

Department of Agriculture investigating uptake of CO_2 from synthetic and flue gas sources and its commercial and environmental viability [48]. The technical feasibility and economic viability of integrating a micro-algal cultivation system with a coal fired power plant was investigated [70], using a bench scale system as a test rig. An artificial flue gas (12% CO_2; 5.5% O_2; 423 ppm SO_2; 124 ppm NOx) based on the composition of a North Dakota power station boiler was produced and sparged into a bio-reactor tank. Two species of algae were cultivated, *Monoraphidium* and *Nannochloropsis*, both of which grew successfully under the administered conditions. It was reported that growth rates of the microalgae varied between 15 to 25 g/m^2/day and contained 41% protein, 26% lipid and 33% carbohydrate [70].

Research using real flue gases for CO_2 uptake and cultivation of algal biomass has also been conducted by a number of other studies. For example, in the study by Doucha *et al.* [71], *Chlorella* sp. was cultivated using a photobioreactor system with the productivity being investigated alongside the injection of flue gas (6–8% CO_2) from a natural gas boiler. The maximum productivity recorded was 22.8 g/m^2/day. The experiment was also conducted using a control gas (pure CO_2), which resulted in a slightly lower productivity of 22.6 g/m^2/day [71]. As a result it was suggested that 50% of the flue gas could be decarbonised using such a system. Similar studies conducted with the species *Chlorella vulgaris* cultivated in a photobioreactor system using flue gas from a municipal waste incinerator indicated that this strain was tolerant to and grew well with a flue gas concentration of 11% (v/v) CO_2. The biomass grown in the flue gas demonstrated a productivity of 2.5 g/L/day compared to the productivity in the control gas of 1.7 g/L/day. The research also showed almost no difference between the growth rate of control gas with a CO_2 concentration of 2% and 11%. Both studies suggest that the presence of potential contaminants in the flue had little adverse impact upon the algae. The impact of CO_2 injection is difficult to quantify given the limited research particularly for raceway ponds. Table 2-5 below displays values of algal productivity from studies where CO_2 has been injected into the system with varying concentrations of CO_2. All of the studies use a photobioreactor set up with artificial lighting.

Table 2-5 Productivity results for different studies investigating microalgae cultivation in CO_2 enriched conditions

Species	CO_2 (%)	P	Unit	Ref.
Chlorella sp.[1]	Air	0.230	/day	[72]
Chlorella sp.[1]	2	0.492	/day	[72]
Chlorella sp.[1]	5	0.127	/day	[72]
Chlorella sp.[2]	Air	0.248	/day	[72]
Chlorella sp.[2]	2	0.605	/day	[72]
Chlorella sp.[2]	5	0.343	/day	[72]
Scenedesmus obliquus	Air	0.216 0.064	/day g/L/day	[73]
Scenedesmus obliquus	6	0.261 0.085	/day g/L/day	[73]
Scenedesmus obliquus	12	0.249 0.076	/day g/L/day	[73]
Scenedesmus obliquus	18	0.260 0.074	/day g/L/day	[73]
Spirulina sp.[3]	Air	0.317 0.107	/day g/L/day	[74]
Spirulina sp.[3]	6	0.327 0.203	/day g/L/day	[74]
Spirulina sp.[3]	12	0.313 0.173	/day g/L/day	[74]

(Note: [1]Low density inoculation [2]High density inoculation [3]Average values used)

Data from the aquatic species program provides some indication of the effect of CO_2 use in raceway ponds. The data below in table 2-6 is taken from the study by Weismann and Tillett [75] from research conducted in 0.1 ha ponds in New Mexico.

Table 2-6 CO_2 use and corresponding productivity from a 0.1 ha pond [75]

CO_2 Use (m^3/day)	Productivity (g/m^2/day)	Carbon use (%)
13.4	8.3	50
14.6	10.5	82
15.2	9.8	59
19.2	18	88
22.0	19	81

From the studies that have been conducted displayed in the table 2-5 it can be observed that generally productivity increases as CO_2 increases although most species have a point at which the concentration becomes too high and productivity decreases [76]. The optimal CO_2 concentration depends upon the species, from table 2-5 it is clear that the *Chlorella* species productivity peaked at around 2% CO_2

whereas the studies using *Scenedesmus* and *Spirulina* showed a tolerance to higher concentrations [73, 74]. As the studies in table 2-5 were conducted in photo-bioreactors, it is therefore difficult to assume that the same results would occur in large raceway ponds although the data from table 2-5 suggests this is the case. Indeed, many other studies have demonstrated that injection is possible to raceway ponds [14]. As carbon is the main constituent of algal biomass (the molecular formula for green algae is $C_{106}H_{263}O_{110}N_{16}P$ [77]), for every 1 kg of biomass produced, 4.4 kg of CO_2 is taken up. Providing flue gases are applied at an efficient concentration and flow, the productivity of algal biomass should be increased thereby taking up CO_2 that would otherwise be emitted to the atmosphere.

Addition of CO_2 has the potential to improve the cultivation of microalgae, particularly in photobioreactor cultivation. In raceway ponds it is likely that the use of flue gas CO_2 would improve biomass yields although the increase may not be drastic. The extra requirements for infrastructure and pumping may outweigh the benefits of using flue gases as a CO_2 source. There hasn't been much research conducted considering the cost (either economically or energetically) of utilising flue gas as a method for increasing productivity and avoiding CO_2 emissions. Kadam [48] estimated that the energy use of pumping flue gas to ponds would be 22 kWh/t CO_2. If a CO_2 use of 22 m^3/day for a 0.1 ha pond as used by Weismann and Tillet [75] is considered, this would require an energy consumption of 0.96 kWh/day or a power requirement of 0.04 W/m^2. Further research is necessary to provide more accurate indications of the costs and impacts of such a process to determine its worth.

2.3.2 Macroalgae

Similarly to most microalgae, macroalgae are photosynthetic and therefore uptake carbon as they grow. Research conducted investigating the uptake of CO_2 from macroalgae biomass has not received the same degree of attention given to microalgae as the cultivation of macroalgae for biomass is less well researched. The cultivation of macroalgae tends to suit large scale areas more than small-scale cultivation making laboratory studies more difficult, seawater is also clearly a necessity which can limit research. The majority of research considering the carbon sequestration potential of macroalgae focusses on the removal of CO_2 from the

atmosphere [78] however some research has considered the use of more concentrated source of CO_2 [79-81]. Research conducted by Gao *et al.* [79] investigated the effect of elevated CO_2 concentrations on the productivity of *Gracilaria* sp. and *G. chilensis* cultivated in bioreactors. The elevation of CO_2 by 650 ppm and 1250 ppm above atmospheric concentrations increased the growth of *Gracilaria* sp. by 130% and 190% respectively, and 20% and 60% for *G. chilensis.* Research conducted by Israel *et al.* [81] examined the potential to use flue gases for tank cultivation of *G. cornea* in Israel. The results suggested that the use of flue gas from a power plant in Ashkelon enhanced the productivity of the biomass as the growth rate with flue gas was 94.1 % per week and 82.9 % without gas. Flue gas and commercial CO_2 provided similar results.

Research to date suggests that macroalgae can provide a form of carbon mitigation through large scale cultivation. Flue gases can be used for enhancing productivity and reducing environmental emissions. The scale of this method, however, remains small as tank cultivation is an intensive method of production.

2.4 Biomass harvesting

Transferring biomass from the cultivation stage to the processing stage is an important part of any biomass to energy system and one that has presented many difficulties in commercialising algal bioenergy. As with the preceding steps, the harvesting method depends very much upon the species of algae and the method of cultivation.

2.4.1 Microalgae

The harvesting of microalgae has been problematic from the first research conducted until current times [82]. The small cell size of microalgal biomass makes effective harvesting difficult and often highly intensive methods of harvesting are necessary [82]. The method of cultivation of the microalgae makes little difference to the subsequent harvesting method as both types of cultivation considered (PBR and pond) grow algae in suspension.

2.4.1.1 Filtration

Filtration can provide a very low input method of harvesting providing the method is applicable to the species of cultivated biomass. Microfiltration can be used if the cell size of the algae is larger than the pore size of the filter. This however, commonly applies to only a small fraction of microalgae, typically those with a cell size greater than 70 µm (*Sprirulina* and *Spirogyra*).The concept is deemed unsuitable for those with a cell size smaller than 30 µm, which includes many of the commonly researched species (*Chlorella*, *Scenedesmus*, *Botryococcus*) [83]. One of the early researchers considering the use of microfiltration was Friedrich Mohn who compared filtration methods for the recovery of *Coelastrum* [82]. Mohn tested two methods of gravity filtration: a microstrainer and a vibrating screen filter. The microstrainer concentrated the algae by a factor 15 and the screen filter by 60 using 0.2 kWh/m^3 and 0.4 kWh/m^3 of energy respectively. Mohn also tested several pressure filtration devices finding the chamber filter press and the belt press to provide the highest concentration factors, 245 and 180 respectively with energy consumption values of 0.88 kWh/m^3 and 0.5 kWh/m^3 respectively. The belt press provides the greatest concentration factor per kWh of electricity consumed for *Coelastrum* [82]. Another option for filtration is the use of ultrafiltration membranes. Problems with fouling of the membrane and the material cost have, however, largely limited their use [84]. Methods to reduce fouling and minimise costs have been examined in recent research and may yield a suitable algal removal technique in the future [84, 85].

2.4.1.2 Flocculation/sedimentation

Flocculation of microalgae is necessary if the cell size is small and need to be combined to allow successful harvesting. Conventional methods of flocculation using flocculants common to wastewater treatment such as alum, ferric chloride, ferric sulphide, chitosan among other commercial products are likely to provide a more consistent and effective solution to flocculation. Much research has been conducted upon the removal of algae using flocculants with varying degrees of success (Table 2-7). For example, a complete removal of freshwater microalgae was recorded for the species *Chlorella* and *Scenedesmus* using only 10 mg/L of polyelectrolytes while a 95% removal was recorded using 3 mg/L of polyelectrolytes

[86]. A comparative study where alum and ferric chloride were used as flocculants for three species of algal biomass (*Chlorella vulgaris*, *I. galbana* and *C. stigmatophora*) indicated the low dosages of alum (25 mg/L) and ferric chloride (11 mg/L) were sufficient for optimal removal of *Chlorella vulgaris*, while higher dosages of alum and ferric chloride were required for the removal of marine cultures *I. galbana* (225 mg/L alum; 120 mg/L ferric chloride) and *C. stigmatophora* (140 mg/L alum; 55 mg/L ferric chloride) [87]. Additionally it has been reported that the combined use of chitosan at low concentrations (2.5 mg/L) and ferric chloride provided much quicker flocculation of the algal cells, *Chlorella vulgaris*, *I. galbana* and *C. stigmatophora*, and reduced the requirement of ferric chloride . The use of chitosan as a flocculant for the removal of freshwater algae (*Spirulina*, *Oscillatoria* and *Chlorella*) and brackish algae (*Synechocystis*) has been investigated [88], and chitosan has been found to be a very effective flocculant, at maximum concentrations of 15 mg/L removing about 90% of algal biomass at pH 7.0. The use of conventional and polymeric flocculants for the removal of algal biomass in piggery wastewater has been recently investigated [87]: ferric chloride and ferric sulphate were found to be effective flocculants at high doses (150–250 mg/L) providing removal rates greater than 90%; polymeric flocculants required less dosing (5–50 mg/L), although provided lower biomass recoveries; chitosan performed poorly at both low and high dosages for each of the algal species types with a maximum removal of 58% at a dose of 25 mg/L for a consortium of *Chlorella*. One of the disadvantages of flocculation is the residual flocculant in the biomass which may affect downstream processes. For this reason organic flocculants can be preferred as they are less likely to adversely impact biomass processing [88]. Table 2-7 displays the removal rates and dosages for each flocculant type tested in various studies for different algal species.

Table 2-7 Results for the removal efficiency of several flocculants

Flocculant	Algae	Removal (%)	Dosage (mg/L)	Ref.
$FeCl_3$	*Chlorella*	98	250	[89]
$FeCl_3$	*S. Obliquus*	95	100	
	Chlorococcum sp.	90	150	
$Fe_2(SO_4)_3$	*Chlorella*	90	250	
	S. Obliquus	98	150	
	C. sorokiniana	98	250	
Chitosan	*Spirulina, Oscillatoria, Chlorella*	>90	15	[88]
Polyelectrolyte (Puriflocs 601 & 602)	*Chlorella, Scenedesmus*	95	3	[86]

Sedimentation provides the second stage of the flocculation process allowing the biomass to settle. Mohn [82] harvested flocculated biomass in a vertical sedimentation tube which was able to concentrate the biomass to a TSS of 1.5% with a concentration factor of 15. Mohn [82] found the results to be satisfactory however never scaled up the technique. Sedimentation of the biomass requires the biomass to settle which is unlikely to always be the case depending upon the algal species however flocculation should enhance the potential for settlement and also the settlement velocity [53].

Where sedimentation is difficult due to the species of algae it can be possible to use flotation where air is pumped into the bottom of a "flotator" where algal cells attach themselves to the bubbles and rise to the surface [53]. The floating biomass can then be scraped off the surface. According to Shelef *et al.* [53], a slurry with 6% total solids is possible via flotation using most types of algal species. Despite being an effective method of solids removal the energy requirement of dissolved air flotation is a high energy consuming process and is thus not commonly considered for removal of algae.

2.4.1.4 Centrifugation

Centrifugation is a highly efficient method of solid/liquid removal using the different densities for separation As with the other alternative methods, centrifugation was considered a feasible option in early algal biomass dewatering work [53, 82]. Golueke and Oswald [86] investigated various means of dewatering algae further to provide a biomass with a sufficiently low moisture content. One of the methods they looked at was centrifugation and three of the four centrifuges that they tested proved to be extremely effective producing a maximum removal of 79% and a biomass with solids content of 11.5% and maximum of 18.2%. Further research was conducted by Mohn [82] in the area of harvesting algal biomass using centrifugation. This research focussed on suitability of algal strains, cost and energy use. In accordance with Golueke and Oswald, Mohn found centrifuges to be very effective for the removal of *Scenedesmus* and *Coelastrum*, particularly the Westfalia self-cleaning plate separator and the Westfalia nozzle centrifuge [82]. The centrifuges provided biomass with total solids content of 2-22% with a minimum energy consumption of 0.9 kWh per m^3 of algal broth Table 2-8 provides an overview of Mohn's findings indicating the possible harvesting methods, effectiveness, energy requirements and reliability of several harvesting methods. Mohn's results suggest filtration provides the best harvesting strategy in terms of high concentration of solids with low energy requirements [82].

Table 2-8 Harvesting methods, effectiveness and energy requirements [82]

Algae species	Harvesting method	% TSS of concentrate	Concentration factor	Energy requirement (kWh/m^3)	Reliability
Coelastrum	Gravity filtration	6	60	0.4	Good
Coelastrum	Pressure filtration	22-27%	245	0.88	Very high
Scenedesmus, *Coelastrum proboscideum*	Centrifuge (Westfalia self-cleaning)	12%	120	1	Very good
Scenedesmus, *Coelastrum proboscideum*	Centrifuge (Westfalia screw)	22%	11	8	Very good

Despite centrifugation being an effective method of concentrating biomass, the energy requirements are much higher than that of filtration. However clearly the choice of harvesting depends heavily upon the biomass type, if the cell size is large enough, then filtration is likely to be the most effective and economically viable option. Otherwise it is likely that a process stream involving flocculation, sedimentation, flotation or centrifugation is necessary. There is little parallel between the effectiveness of common flocculants for harvesting algae in research conducted. It can be observed that there are many effective flocculants for algae removal however suggested optimal dosages vary significantly between studies. Ferric chloride can be considered a viable option potentially combined with chitosan to improve yield and reduce time and material input. Further research is necessary for individual scenarios to choose the most effective method of flocculation and consequent harvesting.

2.4.2 Macroalgae

The harvesting of macroalgae depends very much upon the method of cultivation and therefore will be dealt with separately in this section for each cultivation method.

2.4.2.1 Bottom harvesting

The harvesting of bottom cultivated macroalgae needs to be conducted by hand by divers if the algae is cultivated below the low tidal mark or without diving equipment providing the cultivation is above low tide. It is understood that mechanical harvesting is not beneficial for continued growth and pulling the thalli by hand is preferable [60]. From personal correspondence with experts in macroalgal cultivation and fisherman, it is understood that two fisherman and two divers can harvest one hectare of bottom culture biomass in one day using a fishing type vessel.

2.4.2.2 Long line harvesting

Harvesting of long-line cultivation can be conducted by several methods. The biomass can either be harvested by removing all of the ropes with the attached biomass or by using a cutting vessel which effectively mows the biomass onto a conveyor belt and stores it on the vessel. Regardless of the method, some form of fishing vessel and crew is necessary to gather the biomass from the off-shore site and transport it on-shore. If the rope collection method is used, the thalli must be cut from the rope following harvesting.

2.5 Biomass processing

Once the biomass has been successfully harvested the processes necessary to recover the energy products from the biomass are required. The biomass processing methods depend upon the desired products and often there are several methods to obtain each product. For both types of algal biomass considered there are certain energy products that are more common due to the relative ease of processing and the value of the product. These energy products will be focussed upon as they currently offer the greatest chance of viable full-scale production. Alternative bioenergy products will however also be considered briefly.

2.5.1 Microalgae

As mentioned in the introductory chapter, microalgae is currently receiving the majority of research over macroalgae and particularly for the production of certain biofuel types: biodiesel, bioethanol and biogas. Each bioenergy product requires a

different series of processes to produce the energy carrier, each stream of processes will be considered as well as a combination of the processes. Figures 2-3, 2-4 and 2-5 display the simplified standard processes necessary for each of the methods of bioenergy recovery.

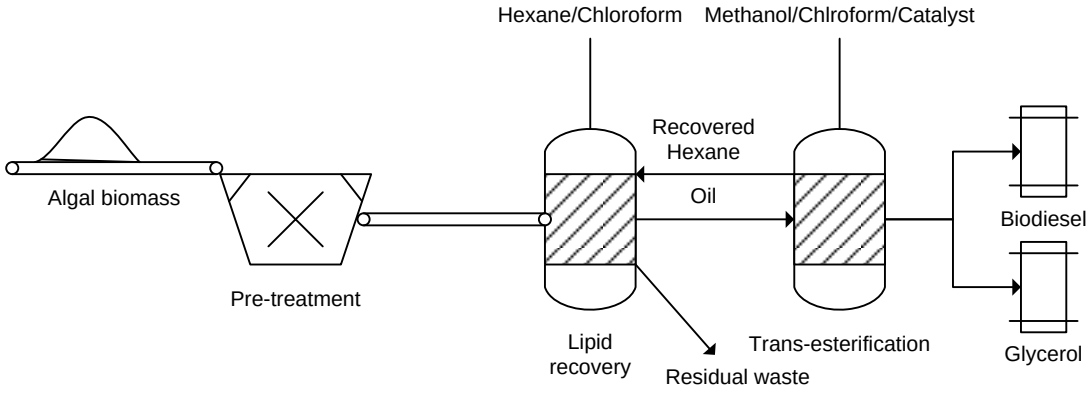


Figure 2-3 Simplified process diagram for biodiesel production

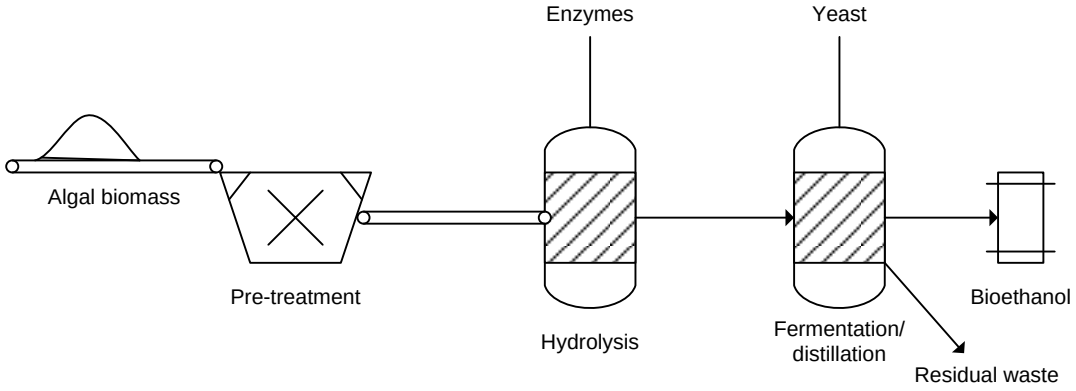


Figure 2-4 Simplified process diagram for bioethanol production

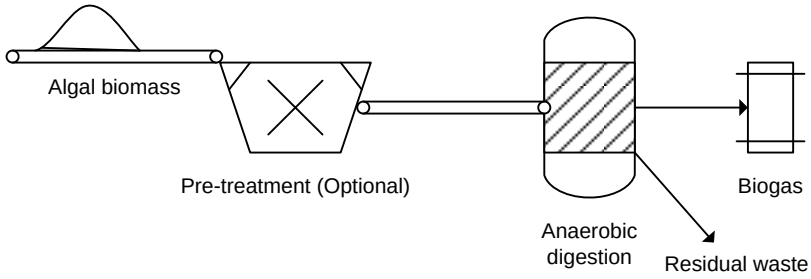


Figure 2-5 Simplified process diagram for biogas production

2.5.1.1 Biodiesel production

Biodiesel is the most common fuel type to be researched as a method of recovering energy from algae due to the high oil content of many algae strains [30, 90, 91]. The production of biodiesel initially requires the extraction of the lipid content of the algal cells. Most researchers follow a standard protocol written by Bligh and Dyer [92] which uses chloroform and methanol as the extraction materials. Prior to lipid extraction the cells must be disrupted to allow access to the oils within the cell. Disruption can be achieved by homogenisation, bead beating, mechanical pressing, microwave treatment, acid/alkali treatment, sonication, lyophilisation and autoclaving among others [93].

Lee *et al.* [94] produced a study investigating the various methods of cell disruption and corresponding lipid extraction efficiencies. They found that for each algal strain (*Botryococcus* sp. *Chlorella vulgaris* and *Scenedesmus* sp.) microwave treatment provided the highest lipid yield. In terms of productive strains, *Botryococcus* spp. provided the highest yield using microwave treatment at 28.6 % lipid recovery from the biomass [94]. Bead-beating however also, almost matched this value. Each of the disruption methods (autoclaving, bead-beating, microwaving, sonication and osmotic shock) produced lipid yields higher than a no-disruption technique.

The next step of the process is the lipid extraction and most studies extract the lipid content of the biomass using a modified version of Bligh and Dyer's method [92]. This requires the addition of methanol and chloroform, typically in proportions of approximately 1:1 methanol:chloroform mixed with the sample also at a ratio of about 1:1 methanol/chloroform mixture:sample [94]. Once the reaction is complete the oil can be separated using a centrifuge or funnelling method as the densities of

the materials differ. Methanol, chloroform and a catalyst (acid or base) are then mixed with oil to allow trans-esterification to occur. The two products from the reaction are methyl-esters (biodiesel) and glycerol. The products are bi-phasic and therefore can be easily separated.

Research within the area is now looking at the possibility of improving extraction of oils from wet biomass which eliminates the energy consumption required for drying of the biomass. It is generally considered that removal of oil from dry biomass is most efficient and practical [91]. Johnson and Wen [95] investigated the use of both wet and freeze-dried algal biomass (*S. limacinum*) for the production of biodiesel. The researchers found that wet biomass produced 20% less fatty acid methyl-esters than the dried biomass, lowering the biodiesel value. Further research has been conducted by Patil *et al.* [96] who conducted experiments producing fatty acid methyl-esters from wet biomass via a supercritical methanol method. The process required only one step for extraction and trans-esterification with addition of methanol at ratio of 1:9 biomass:methanol respectively, a temperature of 255 ˚C and reaction time of 25 minutes. The results showed a Fatty Acid Methyl Ester (FAME) recovery of around 88% from *Nannochloropsis* biomass. The research suggests that high recovery is possible without the energy intensive process of drying and separate lipid extraction. Similarly positive results of direct extraction from wet biomass were produced from Wahlen *et al.* [97] who experimented with direct biodiesel production from various freshwater green algae strains, cyanobacteria and mixed wild algae. More research is required to assess the potential of recovering biodiesel from wet algae in a single stage process, yet the concept appears promising. Energy costs of the process may be higher but this could well be outweighed by the reduced energy cost from drying of the biomass as was calculated by Lardon *et al.* [35] in their LCA of biodiesel from microalgae. This LCA study compared methods of cultivating and processing algal biomass for maximum energy recovery. The authors investigated the energy consumption associated with producing 1kg of biodiesel and found that drying required 81.8 MJ of heat and 8.52 MJ of electricity per kg of biodiesel with no heating requirement for wet biomass. Oil extraction required higher energy consumption for wet biomass than dry but the final energy balance for wet biomass

was considerably positive (105 MJ/kg biodiesel) compared to the negative balance for dry biomass (-2.6 MJ/kg biodiesel).

2.5.1.2 Bioethanol production

An alternative or addition to the production of biodiesel is the production of bio-ethanol from the carbohydrates and starches in the algal cells. Depending upon the strain and composition of the algal species significant yields of ethanol can be produced from algal biomass. Strains with filamentous cells such as *Spirulina* sp. and *Spirogyra* sp. are considered most promising due to the higher percentage of carbohydrate in their make-up. The conventional process of producing bioethanol using hydrolysis and fermentation is well understood for many feedstocks but optimal conversion has not yet been achieved for algal biomass. Similarly to lipid extraction, the first stage in the process is the disruption of the biomass cells which can be carried out using numerous techniques including bead-beating, autoclaving, microwaving and acid or alkali treatment [38]. Once the cells have been disrupted the carbohydrates and starches can be converted into sugars using enzymatic or acid hydrolysis. Following hydrolysis, the sugars are then fermented with yeast (typically *S. cerevisiae* or *S. bayanus*) which will provide a broth of up to 17% (v/v) ethanol depending upon the concentration of sugars (AB Mauri, personal correspondence 18/04/2010). The next step to produce bioethanol is to distil the broth to produce an ethanol concentration of around 98% v/v then further refinement of the ethanol produces a fuel which can be used as an additive to conventional engines or up to a maximum of 85% in specialised E85 engines [98].

As the concept of converting algal biomass into bioethanol is relatively under-researched most studies have simply focussed upon investigating what ethanol recoveries are possible. In an early study by Hirano *et al.* [99] a variety of freshwater and marine algae was selected for testing. *Chlorella vulgaris* was found to contain a high proportion of starch (37%) and a recovery of 65% of ethanol from the starch was obtained using enzymatic hydrolysis followed by fermentation with *S. cerevisiae*. An overall recovery of 24% from the biomass was therefore obtained. Using the strain *Chlorococum* sp. [100], the researchers achieved a conversion efficiency of about 38% of the algal biomass to ethanol which can be considered

promising. However this was an optimal value and no consideration was given to the energy requirement of processing. What is interesting from this research is that when the lipid content of the biomass was recovered prior to fermentation, ethanol yields were far higher. This suggests that biomass could provide both diesel and ethanol, maximising potential recoveries. Nguyen *et al.* [101] found in several studies that yields of up to 29.2% ethanol recovery efficiency were possible using *Chlamydomonas reinhardtii*. The studies mentioned above prove that high ethanol yields from algal biomass are possible but further studies are necessary to assess the viability in terms of energy balance, economics and environmental impacts.

Alternative methods of ethanol production have been investigated which focus upon intracellular ethanol production in which algae produce ethanol under dark, anaerobic conditions. The species which are capable of the process are cyano-bacteria and include the species: *Chlamydomonas reinhardtii*, *Oscillatoria limosa*, *Microcystis, Cyanothece, Cicrocystis aeruginosa* and *Oscillatoria* sp. [102]. The process requires the algae to be cultivated in a closed environment with the addition of CO_2 under which conditions it is believed that concentrations of between 0.5 and 5% ethanol can be produced. Hirano *et al.* [99] investigated this phenomenon using *C. reinhardtii* and *Sak-1* isolated from salt water, and a maximum yield of 1 % (w/w) produced by *C. reinhardtii* was reported. The ethanol-water mix can then be extracted and treated further to produce highly concentrated ethanol for fuel use. The benefits of the process are that no other organisms (e.g. enzymes and yeast) are required for hydrolysis/fermentation and the algae remains unaffected and can continue to grow without a requirement for harvesting. The energy requirements are likely to be lower than those necessary for conventional fermentation of biomass however the two methods need to be directly compared. Although the concept is still very much in the trial phase, a company in the United States, Algenol is currently developing the concept to produce ethanol commercially from *Cyanobacteria* [103]. Intracellular ethanol production is a promising concept. In their study Luo *et al.* [102] show that the whole process provides a positive energy balance with the greatest surplus of energy when the maximum ethanol concentration is produced. Additionally the greenhouse gas emissions compare well to emissions via gasoline

production but to reach 20% of the emissions from gasoline (a government aim) would require further reductions in the process chain.

Bioethanol production from algal biomass is still very much in its infancy, as the concept is proven but the viability is not. Very few studies have considered the inputs and outputs of a commercially sized facility. Due to a lack of data related to large scale operation, accurate modelling remains difficult. Further life-cycle analyses are required to understand the potential of the concept. Post lipid processing and intracellular ethanol production look promising as energy consumption is minimised, further research will establish viability.

2.5.1.3 Biogas production

A simpler method of energy recovery may be facilitated by anaerobic digestion of algal biomass providing a promising source of bio-energy in the form of biogas. The process was considered a potential source of useful energy recovery from algal cultivation near the start of modern research [17]. Anaerobic digestion is a process that has been used for hundreds of years to provide a source of energy from low value organic matter with minor energetic inputs [38]. In the case of algal biomass, all the carbohydrates, proteins and fats can be converted into methane and carbon dioxide, although some components provide greater methane yields than others [36]. It follows therefore that there is slightly less necessity to cultivate particular strains of algae for increased yields.

Table 2-9 taken from a study by Sialve *et al.* [36] displays the methane potential of each biomass component. Research has been conducted investigating the potential of various strains of algal biomass and Sialve *et al.* [36] used the methane potential to calculate yields for a number of strains. Their results can be viewed in table 2-10 which compares theoretical results with experimental results from literature.

Table 2-9 Methane potential from biomass substrate [36]

Substrate	**L CH_4 /g VS**
Proteins	0.851
Lipids	1.014
Carbohydrates	0.415

Table 2-10 Theoretical and actual methane from different algal species

Algae species	Proteins (%)	Lipids (%)	Carbo-hydrates (%)	CH_4 (L/g) (Theoretical) [36]	CH_4 (L/g) (Experimental)	Ref.
Euglena gracilis	39-61	14-20	14-18	0.52-0.8	-	[36]
Chlamydomonas reinhardtii	48	21	17	0.69	0.59	[104]
Chlorella pyrenoidosa	57	2	26	0.8	0.17-0.32 (*Chlorella-Scenedesmus*)	[17]
Chlorella vulgaris	51-58	14-22	12-17	0.63-0.79	0.24	[105]
Dunaliella salina	57	6	32	0.68-0.74	0.44-0.45 (*Dunaliella*)	[36]
Spirulina maxima	60-71	6-7	13-16	0.63-0.74	0.32-0.31 (*Spirulina*)	[36]
Spirulina platensis	46-63	4-9	8-14	0.47-0.69	0.32-0.31 (*Spirulina*)	[36]
Scenedesmus obliquus	50-56	12-14	10-17	0.59-0.69	0.17-0.32 (*Chlorella-Scenedesmus*)	[17]

Table 2-10 suggests that the values of methane yield can vary between species due to compositional make-up and that the yield depends very much upon the growth conditions as this can have a great impact upon the composition of the biomass [36]. Comparing the actual yields with the theoretical yields shows a realistic conversion efficiency loss of about 50% in the majority of cases. It is important therefore that in further studies investigating potential yields, exaggerated or over-optimistic yields are not used as these may not reflect real performance.

As opposed to direct conversion, anaerobic digestion can alternatively be used to recover energy from the waste biomass following extraction of the more valuable components from the biomass cells. In their life-cycle assessment, Lardon *et al.* [35] calculated that the only feasible way of producing a positive energy balance of algal biodiesel was to recover further energy using anaerobic digestion of the residual

waste. In fact, in normal culture conditions, they found that the energy produced from anaerobic digestion would be greater than that from extracted biodiesel. In their investigation of biogas from algae, Sialve *et al.* [36] suggest that at lipid contents below 40% it is unlikely to be worth recovering the lipids using current methods and the biomass should simply be digested to recover the maximum energy yield. In their LCA study of algae digestion Collet *et al.* [33] found the environmental impacts of biogas from algae to be poor in comparison to algal biodiesel using results from the study conducted previously by Lardon *et al* [35]. The study compared the results for 1 MJ of energy produced in a combustion engine. The difference in impacts was mainly due to electricity consumption and the assumption that anaerobic digestion was also applied to the residual biomass following the oil extraction in the biodiesel scenario. The figures used in the aforementioned study provided high values of energy consumption which contrast with those used in other studies [27], the impacts may therefore not be as adverse as suggested. Collet *et al.* [33] concluded that the impacts can be improved with reduced energy consumption and a combined process of lipid extraction and anaerobic digestion may provide the optimal solution.

The biogas produced through anaerobic digestion differs from bio-diesel and bio-ethanol in that it is not a fuel that can be used directly for combustion in vehicle engines. There are two options for biogas, one is combustion within a co-generator to produce electricity with possible heat recovery [106]. The alternative is to refine the biogas removing the CO_2 and the methane can then be used as a fuel within a gas engine [107]. Further energy is required to upgrade the gas to a useable transport fuel and this is often ignored in studies where the energetic content of the gas is only considered. Further research is necessary to investigate the impact of downstream processing if comparison to the alternative biofuel types as a transport fuel is desired.

Anaerobic digestion is one of the methods of recovering energy that seems to provide a positive net energy balance due to the low inputs required [35]. The results may however be optimistic as real yields are much lower than theoretical calculated yields [36]. Additionally the biogas may require further processing to be useful as a fuel and this will affect the energy consumption and environmental impacts. Nevertheless

the process is capable of recovering energy from all strains of algae regardless of the composition and therefore can be very useful as part of a flexible approach.

2.5.1.4 Alternative bioenergy production

This section covered the main energy recovery processes that are currently being researched however there are other energy products worth considering. Alongside ethanol production, it is possible to produce acetone and butane using specific strains of bacteria. Researchers at Utah State University produced Acetone, Butanol and Ethanol (ABE) from algae using the bacteria *Clostridium saccharoperbutylacetonicum N1-4* [108]. The maximum recovery of ABE was 0.311 g/g of biomass. The algae was collected from the local wastewater treatment plant and was a mix of species but mainly *Scenedesmus, Chlorella, Ankistrodesmus, Micromonas,* and *Chlamydomonas*.

Microalgae can be converted to several bio-energy products through pyrolysis of the biomass producing biochar, bio-oil and syngas. The production of biochar has recently been considered a method of carbon sequestration as organic material which has removed atmospheric carbon is pyrolysed and much of the carbon is locked into the biochar which is then not re-released [109]. Bio-oil and syngas are both energy carriers, their proportions of the final product depends upon the heating characteristics of the pyrolysis process. Slow pyrolysis (400°C, reaction time of several hours) tends to favour the production of biochar and fast pyrolysis (500°C, reaction time of several seconds) favours the production of bio-oils and gases [110]. Miao *et al.* [111] yielded 18 and 24% of bio-oil from *Chlorella prothecoides* and *Microcystis aeruginosa* respectively. The oil had a higher heating value of 29 MJ/kg. The use of pyrolysis as a process to recovery energy from algal biomass has not been implemented on a large scale as the high moisture content of the biomass necessitates drying or longer pyrolysis times increasing energy consumption. In their research, Porphy and Farid [112] found that through pyrolysis of *Nannochloropsis* sp. it was possible to recover 14.1 MJ/kg of dry solids. The drying and pyrolysis steps consumed 11.22 MJ/kg of dry solids suggesting a positive energy balance of 2.88 MJ/kg of dry solids. The energy consumption of cultivation and harvesting was not included, however, which would most likely lead to a negative energy balance.

2.5.2 Macroalgae

There is a great difference between the biomass characteristics of micro and macroalgae, thus the potential bioenergy recovery methods are different as a result. Indeed, not only do characteristics vary between micro and macroalgae, the characteristics between the different macroalgae species vary significantly. The species here are broadly grouped into green, red and brown macroalgae. Each of these species groups have more similarity to higher plant types than microalgae and typically contain more carbohydrate content than oil-rich lipids [63]. The carbohydrates however differ between groups and are often unique to specific species. Brown algae typically contain laminarin, mannitol and alginate, green algae contain starch, cellulose, ulvan and red algae contain carrageenan, agar cellulose and lignin [19, 63]. Due to the high carbohydrate and low lipid content of macroalgae the majority of research has focussed upon the production of bioethanol and biogas.

2.5.2.1 Bioethanol production

The majority of research investigating the production of bioethanol has focussed on brown algae, probably due to its relative abundance and ease of cultivation. Particular species which have received attention are *Saccharina latissima* [113], *Laminaria hyperborean* [114], *Laminaria digita* [64] and *Saccharina japonica* [115]. The conversion of macroalgal biomass to bioethanol is not as simple as the conversion of some biomass types such as sugarcane and corn because of the complex carbohydrates contained within the biomass [114]. Some of the unique polysaccharides found in brown seaweeds such as mannitol and laminarin require specific enzymes to convert these polysaccharides to simple sugars which can be used by yeast to produce bioethanol. An ethanol producing bacterium called *Zymobacter palmae* has been reported as having the ability to ferment many different types of sugars including mannitol [114]. The same study however also confirmed that *Z. palmae* was unable to use laminarin. Further work however conducted with an alternative yeast *Pichia angophorae* showed that this particular species appeared to use both the laminarin and mannitol content of the biomass for ethanol production. Using *P. angophorae* a maximum ethanol recovery of 0.43 g/g substrate was recorded and 0.38 g/g mannitol for *Z. palmae.* Using the same strain of yeast (*P.*

angophorae), Adams *et al.* [64] managed to produce 167 ml of ethanol per kg (132 g/g) of *Laminaria digita* using the enzyme laminrase for pre-treatment. Another research group managed to recover high yields of ethanol by discovering a method to use DNA from a bacterium called *Vibrio splendisus* alongside *Escherichia coli* allowing the conversion of alginate in *Saccharina japonica* to ethanol [115]. The group managed to recover an ethanol yield of 0.281 g/g biomass.

Bioethanol from red macroalgae has received slightly less attention that of brown probably largely due to comparatively less prevalence. Wang *et al.* [116] investigated the two stage hydrolysis and fermentation of the invasive red algae *Gracilaria salicornia* finding a recovery of 79.1 g of ethanol per kg of biomass. Kumar *et al.* [117] investigated the potential for ethanol recovery following agar removal from *Gracilaria verrucosa*. The researchers used enzymatic hydrolysis with cellulase and β-glucosidase to release the sugars in the biomass yielding 0.87 g sugars/g cellulose. The sugars were fermented using *Saccharomyces cerevisiae*, a common yeast, to recover 0.43 g ethanol/g sugars.

Ulva has received the most research for bioethanol production in terms of green macroalgae as it is one of the most common species of green macroalgae. *Ulva* is a relatively fast growing macroalgae and therefore has been considered as a potential source of bioenergy [118]. Several studies have considered bioethanol production, Masutani and Yoza [119] enzymatically hydrolysed a batch of *Ulva fasciata*, sampled locally in Hawaii, using a cellulase enzyme. The hydrolysate was fermented with *S. cerevisiae* yielding the equivalent of 126 litres of ethanol per tonne of biomass. The researchers believed this to be about 43% of the potential of the biomass given the characteristics. Similar experiments have been conducted by the Danish Technological Institute looking at bioenergy recovery from *Ulva lactuca*. The biomass was hydrolysed with a number of commercial enzymes and fermented with *S. cerevisiae*. The greatest yield produced was 0.141 g ethanol per gram of dry biomass [118]. Table 2-11 displays the bioethanol yields recovered from various studies for a number of different species and processing methods.

Table 2-11 Bioethanol yields recovered from macroalgae from a variety of studies

Species	Materials	Bioethanol yield (g ethanol/g biomass)	Ref.
Laminaria digitata	*Laminarase* (pre-treatment) and *Pichia angophorae*	0.218[1]	[64]
Saccharina japonica	*Vibrio* splendisus and *Escherechia coli*	0.281	[115]
Gracilaria salicornia	Sulphuric acid and *Escherechia coli*	0.079	[116]
Ulva lactuca	Enzyme mix and Saccharomyces cerevisiae	0.141	[118]

(Note: [1]Assuming a bioethanol density of 0.789 g/ml)

2.5.2.2 Biogas production

As a relatively simple and low intensity method of energy generation, anaerobic digestion has been researched as a method of recovery of energy from macroalgal biomass. Macroalgae has been considered as a potential feedstock for methane generation since the late 1970s where the anaerobic digestion of *Macrocystis pyrifera* was investigated [120]. *Macrocystis* is the brown algae which has been studied most and many studies have obtained high yields of methane recovery [121]. In early research Ghosh *et al.* [122] recovered around 281 cm^3/g VS from marine giant kelp (*Macrocystis pyrifera*) finding that the algin and mannitol in the biomass were highly biodegradable. Higher methane yields were recovered by Chynoweth *et al.* [121] where *Macrocystis* yielded methane values of between 0.39 and 0.41 L CH/g VS, a higher yield than *Sargassum* and *Laminaria* which produced maximum methane yields of 0.38 and 0.28 L CH_4/g VS respectively. *Saccharina lattisima* has also been investigated as a biogas source with a recovery of 0.268 L CH_4/g VS having been reported [123].

More recent large scale tests have been conducted in Japan by Matsui *et al.* [124] examining the generation of biogas from brown algae (*Laminaria* sp.) and green algae (*Ulva* sp.). The loading rate of the seaweed ranged from 0.2 to 1 tonne of biomass per day. The digestion was thermophilic with the temperature kept at 55°C and a retention time of between 15 and 25 days. The greatest recorded yield of methane from *Laminaria* sp. was 0.22 L/kg of biomass corresponding to a biogas

yield of 0.329 L/kg. The ash content of the biomass was determined to be 62.7%, the yield was therefore 0.351 L CH_4/kg VS. The yield of methane recovered from *Ulva* sp. was less with yields between 0.15 and 0.17 L CH_4/kg biomass or 0.18-0.20 L CH_4/kg VS given the determined ash content of the biomass.

Other studies which have considered *Ulva* biomass are Wise and Ryther [125] who investigated the recovery of methane from *Ulva lactuca* and also from the red algae, *Gracilaria ceae* recording yields of 0.190 m^3 and 0.114 m^3 of methane per kg of volatile solids respectively under mesophilic conditions (37°C). A similar and more recent study by Costa, Gonclaves *et al.* [126] also investigated biogas from both *Ulva* and *Gracilaria* biomass. The authors again found *Ulva* to be a better producer of methane, producing 196 L CH_4/kg VS compared to 182 L CH_4/kg VS from the *Gracilaria*. These experiments were conducted under mesophilic conditions (37°C). Slightly higher yields from *Gracilaria tikvahiae* were recorded by Habig *et al.* [127] with a maximum methane yield of 0.23 L CH_4/kg VS under mesophilic conditions (32°C). The authors also digested *Ulva* biomass and similarly to the other studies where both species have been investigated the maximum methane yield was higher at 0.33 L CH_4/kg VS. Recent investigations by Bruhn *et al.* [128] recorded a maximum methane recovery of 0.271 L CH_4/kg VS. Table 2-12 displays the methane yields obtained from various studies for a variety of algal species and processing conditions.

The range of methane yields varies greatly between species of macroalgae but also within species depending upon the conditions of the study. Nevertheless it is possible to note which species are more favourable towards anaerobic digestion. The studies reported here suggest that brown macroalgae contain the most anaerobically biodegradable material with methane yields varying from around 0.268 L CH_4/kg VS up to 0.41 L CH_4/kg VS. Additionally it appears that most of the species of macroalgae which have been tested are conducive to the production of methane. Methane yields from red algae are comparatively low with a maximum yield of 0.230 L CH_4/kg VS being recorded, slightly over half the maximum yield of *Macrocystis pyrifera*. Green algae, mainly *Ulva* sp., has produced a range of yields

from 0.18 to 0.33 L CH_4/kg VS suggesting it provides a better feedstock than red algae but less favourable than brown.

Table 2-12 Biomethane yields recovered from macroalgae from a variety of studies

Species	Yield (L CH_4/kg VS)	Conditions	Ref.
Brown algae	0.268-0.41		
Macrocystis pyrifera	0.281 0.39-0.41	Mesophilic	[122] [121]
Laminaria sp.	0.28 0.329	 Thermophilic (55°C)	[121] [124]
Sargassum sp.	0.38		[121]
Saccharina latissima	0.268	Mesophilic (37°C)	[123]
Red algae	0.114-0.23		
Gracilaria ceae	0.114	Mesophilic (37°C)	[125]
Gracilaria tikvahiae	0.230	Mesophilic (32°C)	[127]
Gracilaria sp.	0.182	Mesophilic (37°C)	[126]
Green algae	0.18-0.33		
Ulva sp.	0.18-0.20 0.196 0.33 0.271	Thermophilic (55°C) Mesophilic (37°C) Mesophilic (32°C) Thermophilic (52°C)	[124] [126] [127] [128]

2.5.2.3 Alternative bioenergy production

Given the low lipid contents of macroalgal biomass there has been limited research investigating oil product extraction although some studies have been conducted. Maceiras *et al.* recovered a maximum of 11.5% biodiesel from a mixture of macroalgal species found on the Galician coastline [129]. Although the study demonstrates the conversion is possible, the yields are low for such an energy intensive process. Sialve et *al.* [36] suggested in their study that an oil content lower than 40% was unlikely to be worthwhile recovering from microalgae when anaerobic digestion was to be used. A similar maximum concentration of biodiesel was recovered by Suganya and Renganathan [130] from *Ulva lactuca*, their study yielded 10.88 % biodiesel from biomass.

Macroalgae has been tested for its potential as a feedstock for bio-oil production from pyrolysis by Bae *et al.* [131] The researchers tested 4 different feedstocks from marine macroalgae recovering a maximum bio-oil yield of 47.4% from the red algae,

Porphyra sp. *Undaria* sp., a brown algae, produced 45.8% bio-oil following acid washing pre-treatment. Untreated *Undaria* produced 39.5% bio-oil and *Laminaria* sp., 37.5%. Maximum yields were obtained at a temperature of 500°C. Despite high bio-oil recovery the authors conclude that the use of the macroalgae does not look promising due to the high nitrogen content of the bio-oils produced. Direct combustion of macroalgae has been considered [132] however the authors conclude that due to the high moisture content, low calorific value, high chlorine and high ash contents of the biomass there is little value to thermochemical conversion of the biomass.

2.6 Limitations

Despite the great potential for the utilisation of both microalgae and macroalgae for their use as feedstocks for recovery of energy there are very few examples of large scale projects of either. The systems to produce energy from these feedstocks still need to overcome barriers limiting the large scale development of such systems. This section will consider the current limitations hindering the commercialisation of bio-energy recovery from algal biomass.

2.6.1 Microalgae

The details of the typical methods of microalgal biomass production have been covered extensively in this chapter, the methods employed have a great bearing upon how viable the overall system can be. The two main methods of biomass generation are open cultivation in raceway ponds and closed cultivation in photobioreactors. In general photobioreactors provide the best method to cultivate specific species of algae without interference from foreign bacteria yet the intensity of cultivation is very high due to the infrastructure and constant gas and water pumping necessary. As a result unless highly efficient designs of PBRs are produced requiring little energy input and few energy intensive and costly materials it is unlikely that they will play a main role in large scale generation of algal biomass. Their use however is highly beneficial for laboratory research and initial cultivation of specific strains. Open ponds appear to provide the best option for large scale biomass cultivation due to the low impact characteristics of construction and operation. Nevertheless this method of cultivation currently has fundamental problems, the main issues being:

- Poor species control
- Required land area
- Water requirement
- Material use
- Poor environmental control

Each of these issues affects the viability of bio-energy recovery from microalgal biomass whether it's due to a resulting reduction in biomass productivity or high embodied energy in material inputs. As a result of the method requiring open cultivation there is a high risk of species invasion providing a non-localised strain of algae is used for initial inoculation. This has been reported as occurring in many instances where the use of ponds has been investigated [14, 47, 133]. This ultimately means that the final type of biomass cultivated largely depends upon what the local strains of algae are. Using a non-selected strain of algae is likely to lead to lower than anticipated yields and a recovered biomass with less value than some selected strains. The downstream processing methods must also be adapted to the species of biomass which becomes dominant as each processing method requires specific characteristics. For this reason it is difficult to design a system using set assumptions (productivity rates, species characteristics) as any assumption need to be made with consideration for the specific location which would require unique studies.

It has been shown that productivity in ponds is greatest at a relatively low depth allowing efficient use of sunlight and gas transfer. Studies suggest the optimum depth of water to be around 0.25 to 0.5 m [14, 37]. The obvious negative of such low depths is the large areas required to produce sufficient quantities of biomass to justify the development of such a system. The area required naturally depends upon the availability of water resources which depends upon local conditions and the goal of any proposed development. The concentration of microalgal biomass cultivated in ponds is generally much lower than that which can be accommodated in photobioreactors [37]. As a result a high volume of water is required per mass of microalgae produced. Given the water scarcity of many parts of the globe, particularly areas in which microalgal cultivation has received most consideration (New Mexico [14], California [134] and Israel [47]), the high water use can be a

major limiting factor. It is estimated that by 2030 half of the world's population will be living in areas of water stress [135]. The use of freshwater has recently been considered largely unfeasible for large scale cultivation projects due to the value placed upon this resource. The obvious solution is the use of wastewater for the dual benefit of a readily available supply of water and as a method of contaminant removal from the wastewater. The cultivation of microalgae in a variety of wastewater streams has been proven possible by a number of studies with most research focussed on municipal wastewater [136-138] and agricultural wastewater [139, 140]. The acceptability of the wastewater is determined by the extent of contamination with many industrial wastewaters containing too high a concentration of toxic contaminants and too low a concentration of nitrogen and phosphorous for effective growth of algae. The potential to use municipal and agricultural wastewater is relatively well accepted particularly for use as a polishing pond to remove the nitrogen and phosphorous present in the water prior to discharge. Using *Neochloris oleoabundans* cultivated in artificial wastewater, Wang and Lan [136] found that the algae removed 100% of the phosphorous for every concentration tested and 78-99% of nitrogen removal depending upon the initial concentration. Woertz *et al.* [141] cultivated a mixed inoculum from a local wastewater treatment plant in municipal wastewater in a photobioreactor set up achieving greater than 99% removal of nitrogen (ammonia) and phosphorous (phosphate) over a three day hydraulic retention time. These experiments used artificial CO_2 to stimulate growth. When only air was used, 84% of nitrogen and more than 99% of phosphorous was removed. The effectiveness as a method of nutrient removal is highly dependent upon the productivity of the biomass which requires a set range of temperature, sunlight, CO_2 and nutrients. It is possible that any of these conditions could become a limiting factor therefore reducing the efficacy of the concept as a viable form of nutrient removal. Most studies for example have noted a distinct drop in biomass productivity during the winter months which would undoubtedly lead to poor nutrient removal in wastewater and potentially the requirement for an alternative treatment method.

In addition to nutrient removal, microalgae can also have the capacity to remove heavy metals from wastewater. Wang *et al.* [142] cultivated *Chlorella* sp. in various municipal wastewaters contaminated with heavy metals using a photobioreactor set

up. The results showed removal rates of between 65.5-87.3% for aluminium, 22.6-95.4% for cadmium, 98.3-100% for iron, 80-98.4% for magnesium, 98.2-100% for manganese and 56.5-81.2% for zinc over a period of nine days. Although conducted in a photobioreactor under controlled conditions, some degree of metal uptake will also be present in a raceway pond system thereby providing an additional service.

The environmental temperature has a great impact upon the productivity of most species of algae. Algae can generally survive at temperatures between 10 and 30 °C although temperatures around 20 °C tend to be favourable for high productivity [143]. In ponds, temperature control is difficult and the algae must therefore be able to thrive under the environmental conditions. In most countries with distinct seasons, the productivity will likely be reduced when the temperature falls too far below or rises too high above the optimal temperature. This was observed with a decrease in productivity of algal biomass in outdoor ponds in Israel [47]

The material requirements for pond cultivation of algal biomass are less intensive than those necessary for photobioreactors but can still be a considerable burden to the production process [37]. Raceway ponds can be constructed from a variety of materials. However due to the cost effectiveness of the material and ease of production, concrete or brickwork is generally the preferred option. Most of the large scale ponds which have been tested are of similar sizes with standard dimensions of 100 m by 10 m [14, 37]. Due to the potential losses of seepage through concrete blocks a thin liner is usually used. In work conducted as part of the aquatic species program however, the researchers found that the use of a liner had no beneficial impact upon the productivity of the algal biomass. Given the potentially large areas the use or otherwise of a plastic pond liner could have a significant bearing on the viability of a raceway pond system.

There are few other infrastructure requirements for raceway ponds. The most prominent of these following the structure of the pond is the paddlewheel for mixing and a carbon dioxide sump if used. Paddlewheels continue to be considered the most practical and cost effective method of water mixing in the raceway pond design. There haven't been many instances where the energy consumption of paddlewheels have been considered. In their LCA study, Clarens *et al.* [27] calculated the power

rating of a single paddlewheel to be 0.037 kW based on a study by Moulick and Mal [56] investigating the performance of a double hub paddlewheel. Clarens *et al.* [27] assumed one paddlewheel was required per 100 m^2 and would be in operation 24 hours per day. In the New Mexico test facility as part of the Aquatic Species Program, the researchers estimated the energy consumption to be 0.04 W/m^2 or 40 W per 1,000 m^2 pond. If CO_2 is injected into the pond for increased yields the injection of the CO_2 requires a carbonation sump as used in the New Mexico test facility where it was estimated that practically 100% of the CO_2 was utilised in the pond. For the 0.1 ha test facilities the CO_2 use ranged from 13.4 to 22 m^3/day. The sumps were at a greater depth in the pond than the overall depth at between 0.6 and 0.9 metres and the gas injected against the current to increase utilisation efficiency. CO_2 can generally be used from power stations where the flue gases contain CO_2 concentrations up to about 13% [14]. Flue gases from power stations have been proven to be usable by different species of microalgae [48, 69, 71, 73]. The obvious limitation of using flue gases is the availability of flue gas which requires a form of power station to be situated near the cultivation ponds. Supposing the biomass is processed and the energy product potentially used for energy generation on-site there is the possibility for recovering CO_2 from these processes. Kadam [48] estimated the cost of CO_2 injection to be 0.022 kWh/kg CO_2.

If fertiliser is required, the economic, energetic and environmental costs can severely impact the overall sustainability due to the intensity of fertiliser production. Obviously the use of fertiliser is preferably avoided and studies have shown that the use of the nutrients in wastewater is a far more sustainable source [27]. Nevertheless the problem with the use of wastewater as a source of nutrients for generation of biomass is the inconsistency of nutrient loading and the non-optimal concentrations of the nutrients. Algae require a specific proportion of nitrogen and phosphorous which is unlikely to be accommodated, therefore for optimal growth and complete removal of the nutrients it would be necessary to supplement the cultivation ponds with the limiting nutrient.

Once the algal biomass has been cultivated, its removal is clearly necessary to allow processing and energy recovery to be conducted. As algae cultivated in open ponds

tend to be highly dilute, the method of harvesting can be a great contributor to the overall intensity of the biomass production [82]. Centrifugation is largely considered the most effective method of microalgal recovery. For dilute concentrations, however, the cost is prohibitive, the biomass must first be more concentrated. The conventional method to achieve this is through flocculation and settling, with many types of flocculants showing the capacity to allow the flocculation of microalgal biomass. Typical flocculants are grouped into inorganic (e.g., ferric chloride, ferric sulphate, alum) and organic flocculants (e.g., chitosan) [87]. The efficacy of both types of flocculants have been demonstrated in a number of studies with removal rates of microalgae above 90% for most studies and some achieving almost 100% removal [88, 144]. Nevertheless despite the effectiveness of removal with flocculants there are certain drawbacks, namely the potential toxicity of the flocculant and the cost of flocculant production. The presence of certain metals in algal biomass may well affect subsequent processing of the biomass particularly if bacterial processes are necessary [145]. Chitosan is considered a relatively cheap, organic flocculant with a high removal efficiency but is limited to freshwater [145]. Flocculation is a relatively cost effective method of allowing concentration and settling of algal biomass however the type of flocculant which can provide adequate results depends upon the species of algae. The required dosing may be high and depending upon the flocculant used may provide issues regarding downstream processing.

Alternative methods to the use of traditional flocculants have recently received attention such as the use of auto-flocculating microalgae which Salim *et al.* [146] demonstrated improves the sedimentation efficiency of typical non-flocculating species such as *Chlorella vulgaris*. Although it can provide improved sedimentation this method of flocculation is not as effective as using conventional flocculants, the cost however may be less. Electrolytic flocculation has been shown to provide a high recovery efficiency with a relatively low electricity consumption [147]. Despite promising results this technology has not seen widespread uptake on any scale.

Once the biomass has settled it requires further dewatering which in most studies is assumed to be conducted using centrifugation due to the effectiveness of the method. Centrifugation however comes at a high price in terms of the energy use. In early

work by Mohn [82], several centrifuges by different manufacturers were tested and the energy consumption varied from 0.9 to 8 kWh/m^3 for those which provided at least a good level of reliability. Centrifugation is considered a necessity in most studies [10, 33, 45] however the inclusion increases the proportion of energy consumption during the harvesting process greatly. Belt and filter presses are an alternative [35], yet the concentration of the biomass is not quite as high as that of centrifugation and their energy consumption is also high [82].

The next step of the biomass processing depends upon the method of energy recovery as to whether the biomass requires drying or not. The process of biomass drying is ideally avoided as it's a highly energy intensive process given the moisture content of the recovered biomass. In their LCA study, Lardon *et al.* [35] calculated that the drying process required more than 10 times as much energy as the oil extraction process. Oil recovery and transesterification can be conducted with wet biomass but the efficiency is reduced and the hexane requirement is much greater [148] and the volume that can be recycled is reduced. The extraction of oil from the biomass requires treatment with hexane. The hexane, however, can be recycled through distillation with a small loss. The transesterification process requires the use of methanol and chloroform according to the method of Bligh and Dyer [92] for the generation of methyl esthers. The reaction also requires relatively high temperatures necessitating heat generation and therefore energy consumption. Given the high material and energetic inputs to the transesterification process it is necessary for the biomass to have sufficient oil content to justify extraction and esterification. In their study, Sialve *et al.* [36] concluded that in order to be energetically worthwhile, the algae being processed should contain at least 40% lipids. Given that most non selected species of algae contain less than 40% lipid content, the idea to convert those species which come to naturally dominate in constructed raceway ponds may be jeopardised by the low oil content.

Limited studies have considered converting algal biomass to ethanol but dry biomass is usually used for more conventional feedstocks such as corn making mechanical cell disruption easier. In most studies examining bioethanol from microalgae the biomass is pre-dried [100, 149]. The assumption is that biomass drying would be

necessary in a large scale system thus incurring the associated energy costs. As a result of fermentation and distillation of algae not having been practiced on a large scale the energy and resource requirements are largely unknown. Despite the success of many of the studies that have investigated bioethanol production from microalgae, it must be noted that the methods used are potentially highly energy intensive. Enzymes are commonly used for the hydrolysis stage where enzyme production has an associated energy and environmental cost which can reduce the viability of the process [150]. Similarly the use of acid hydrolysis requires the production of acid, an energy consuming process and a harmful product. Additionally the production of yeast to convert the sugars to ethanol requires a certain amount of energy and resource consumption, albeit a slightly smaller one than enzymes [150]. Both fermentation and distillation processes require heat to allow the reactions to take place reducing the overall potential energy balance of the concept.

The process of anaerobic digestion is one that does not require pre-drying and therefore the drying costs can be avoided. For effective methane generation some pre-treatment methods that disrupt the algal cells are, however, beneficial [36]. In comparison to biodiesel recovery and fermentation/distillation to ethanol, the process of anaerobic digestion requires much fewer inputs both materially and energetically. The main limitation associated with anaerobic digestion of microalgal biomass is whether the methane yields produced can justify the cultivation and harvesting of the biomass as well as the potential upgrading of the biogas for use a transport fuel or alternatively for electricity generation.

Clearly there remains many barriers to the successful implementation of a large scale system to cultivate and process microalgal biomass to a form of energy. In each process step there are issues needing to be overcome. The most effective method of cultivation (in terms of low inputs) cannot sustain specific species and therefore non-selected strains tend to dominate. Non-selected strains are unlikely to have the ideal characteristics for the subsequent processes, effective harvesting and high energy recovery yields. Harvesting method are species dependent with few species suitable for microfiltration. Flocculation is generally effective in allowing settlement however inorganic flocculants are undesirable. Centrifugation provides almost guaranteed

dewatering but the energy cost is very high. Depending upon its requirement, drying of the biomass requires a further energetic input to the system particularly as the moisture content of micralgal biomass is high reducing the overall energy balance. Algal biomass can be converted to different energy products. The product which has been given the most attention is biodiesel yet the energy requirements for extracting the oil and converting to diesel are very high which may make the process unfeasible if oil contents are too low. The fermentation and distillation of biomass to bioethanol is possible yet there are a limited number of studies considering the viability on a large scale. The material and heating requirements of the fermentation/distillation process are high and may make conversion unfavourable. Anaerobic digestion of the biomass is a simpler process but yields a less valuable energy carrier which would require some form of upgrading which may outweigh the benefits of its production.

2.6.2 Macroalgae

Macroalgae as a source of energy recovery have received much less attention than microalgae and therefore the limitations of their development are at a more fundamental stage. In contrast to microalgae the cultivation differs greatly depending upon the species considered. Species that are currently cultivated on a large scale are done so either by bottom-planting, long line cultivation or in artificial ponds [19, 60, 151]. Prior to each of these methods initial biomass preparation is necessary either for establishment of thalli which can be planted or the production of spores for inoculation. These preparation methods are highly labour intensive. Depending upon the culture method the placing of the biomass can be problematic. Bottom planting requires workers to place biomass in the sea floor either at areas under low tide or areas between low and high tide [60]. Even more automated methods of conducting this require a lot of manual labour and potentially diving equipment and a small fishing vessel. Long-line cultivation is carried out where ropes can float on the coastline, areas containing structural ropes and anchoring are required with which to attach the inoculated ropes. Similarly manual labour is required to attach the long lines using a fishing type vessel and diving equipment. The harvesting of the biomass requires a similar intensity of input to recover the biomass or ropes carrying the biomass.

During the cultivation phase there is little input required for the offshore cultivation methods as the environment provides the necessary inputs (nutrients, CO_2, sunlight). There are however many problems that can be encountered during the cultivation phase. Given that the offshore method of cultivation is entirely open to environmental conditions, the productivity of the biomass can be greatly affected by adverse conditions. One of the most common hindrances is the occurrence of organismic infections or epiphytism [61]. Foreign plant species or parasites can often attach themselves to biomass that is being cultivated reducing productivity rates or destroying the crop entirely. Offshore cultivation techniques are also highly susceptible to adverse weather conditions which may destroy or uproot crops. Unseasonably cold temperatures or a lack of nutrient concentration in the water may also reduce expected productivity rates.

Tank or pond cultivation of macroalgae has an entirely different set of limitations in relation to offshore cultivation. The investment in infrastructure in terms of cost, materials and energy is far greater as structures are required to contain the seawater and biomass. The water must also be kept in motion to allow the biomass to receive a constant supply of sunlight and nutrients. This method of cultivation is much more intensive than the offshore method whilst producing greater masses of biomass for a given area. The energy requirements are also much higher.

Once the biomass has been harvested the options for recovery of energy are constrained by the characteristics of the biomass. Macroalgae typically contains little oil fraction and therefore extraction is not generally considered viable [63]. Processes which can use the carbohydrate fraction are therefore favoured, yet the carbohydrates contained within each macroalgae species are particular and to allow their conversion to a more readily accessible form often requires unique treatment. To produce bioethanol the carbohydrate content must be hydrolysed to produce simple sugars which can undergo fermentation. Specific enzymes have been developed which are able to utilise many of the previously intolerant fractions of macroalgal biomass. The overall energy balance of the concept however has not been well studied and large energy inputs are expected to pre-treat the biomass and heat the fermentation and distillation processes. Apart from the high energy costs, a source of freshwater is

likely to be necessary to wash the biomass prior to pre-treatment to remove sodium which may inhibit subsequent processing.

The generation of methane suffers many of the issues that limit the recovery of ethanol in that many of the structural components cannot be broken to allow bacterial degradation. As biogas is not considered such a high value product in comparison to bioethanol, intensive pre-treatment methods are unlikely to be cost-effective or sustainable as part of its production. Low methane recoveries are likely for those species which contain carbohydrates that cannot be degraded which restricts species that can provide an adequate feedstock for methane recovery.

2.7 Improving sustainability

To improve the overall sustainability of cultivating algal biomass and the recovery of energy, all process areas need to be considered. The challenges of producing energy from algal biomass are unique to specific scenarios depending upon location and designs. These limitations however can be broadly defined and approaches to overcome them considered on a general level.

2.7.1 Microalgae

It has already been mentioned that raceway ponds provide the greatest potential for large scale cultivation of microalgae due to the lower material and energy inputs involved. Concrete provides a cheap and relatively flexible material for the basic pond infrastructure, it is unlikely that there is an alternative material that can compete due to costs although materials with a lower environmental impact should be considered. Many studies use a pond liner however due to the intensity of plastic production the use of liners should be avoided. According to studies conducted during the NREL Aquatic Species Program the use of pond liner did little to enhance productivity rates over not using one [14]. Paddlewheels provide a low impact method of mixing the water and algae with few alternative methods which can provide similarly effective mixing with such low energy consumption. The inclusion of a CO_2 sump and injection of CO_2 has been shown to improve productivity in most species of microalgae, however using synthetic CO_2 is unlikely to be a sustainable

solution. Use of a flue gas if available would improve the sustainability of the process as suggested by Clarens *et al.* [10].

Due to the high environmental cost of fertilisers, nutrients must be used that would otherwise be considered a waste stream. Municipal and agricultural wastewaters provide the main nutrients necessary for algal cultivation and the cultivation provides the added benefit of cleaning up the wastewater following more conventional treatment processes. Ideally the cultivation pond is situated beside a non-toxic wastewater stream allowing a free stream of nutrients and off-setting any nutrient removal process that may have been required otherwise. Additionally depending upon the applicability of subsequent biomass processing any wastewater that is produced can provide an extra source of nutrients and avoid wastewater treatment. Obviously the size of the cultivation system is entirely dependent upon the daily flow of wastewater.

As mentioned previously the control of species in open ponds is difficult as localised species develop naturally [47]. Species could potentially be controlled by covering the ponds with plastic however this would require a large investment in terms of cost and embodied energy. An alternative method of preserving species dominance is to use a species that require extreme environments such as *Spirulina* sp. which requires a high pH environment [152] or to use a salt water species in saline industrial effluent inland. These methods are however highly inflexible and would require the addition of further materials to retain ideal conditions. The alternative is to allow a natural species of microalgae to dominate and adjust the subsequent processing methods to the naturally developed biomass. The species which dominate are highly dependent upon the location of the cultivation system and it is most likely that a mixture of species would become dominant.

The most sustainable harvesting method depends upon what species of algae is cultivated or becomes dominant. The most cost effective and least energy intensive method of harvesting is filtration which should be adopted where possible. Filtration can provide good concentration of biomass providing the main species has a cell size great enough to be separated by the filter. Filamentous biomass is the preferred type for this technique. Flocculation is a possible solution for species which cannot be

removed through filtration and good settlement has been demonstrated following flocculation from a number of studies [146, 153, 154]. Flocculation with chitosan is favoured over non-organic flocculants as a more environmentally innocuous substance which is easily produced. If high biomass solids concentrations are required centrifugation provides the most effective method of dewatering yet the energy cost is high.

The purpose of bioenergy recovery from the biomass is to recover the maximum amount of energy possible with the lowest energy input. Production of biodiesel is limited by the oil content of the algal species, if the content is sufficiently high (at least 40% according to Sialve *et al.* [36]) then the extraction of lipids and conversion to methyl esters is likely to be beneficial. Unless species control is effective, it is unlikely that a naturally occurring species will contain this high a proportion of oil. The production of bioethanol also requires specific contents of biomass but these are contents which can be converted to simple sugars which are generally more abundant in less selected strains of algal biomass. Yields of bioethanol from naturally occurring and non-manipulated strains of algal biomass can potentially be high. However the energy input to the system is important as material and energy use for fermentation and distillation are typically considerable. Anaerobic digestion provides a method of energy recovery which has the ability to convert most of the volatile components of organic biomass. This method of energy generation probably provides the most flexible method of energy recovery as high yields can be recovered from most species of algae. Additionally the energy inputs of anaerobic digestion are low as the moisture content of the biomass can remain high and at mesophilic temperatures (around 30°C) little heating is necessary. Another simple method of energy recovery is simply direct combustion of the biomass which according to Clarens *et al.* [10] may provide the greatest return of energy for the energy consumed.

2.7.2 Macroalgae

The initial intensity of offshore macroalgae cultivation, the preparation of the biomass by either cutting and planting of thalli or inoculation of ropes and offshore placement is largely unavoidable. The main benefit of these processes is the employment prospect on coastal areas.

Cultivation of biomass in tanks is highly energy intensive as the use of air blowers is the common method to allow mixing and access to air. Such a method of cultivation on a large scale is unlikely to be viable for low value energy recovery in comparison to offshore mass cultivation methods. As the offshore cultivation of macroalgae is largely unaided there are little modifications that can be made. The sustainability however can be improved by locating the culture of algae near to a source of waste nutrients such as a fish farm. Nutrient run-off from salmon farming has been shown to improve productivity of the biomass whilst providing a certain amount of nutrient removal from the water [151, 155]. The cultivation of non-native species of algae should be avoided as such species could be more susceptible to infection from local organisms. Epiphytism is likely to occur naturally and is difficult to control, the use of pesticides is not recommended due to the resultant pollution and the low effectiveness of the method in open waters. Perhaps the only method to reduce the impact of epiphytism is to test different species and strains of macroalgae for their tolerance to epiphytes.

Harvesting offshore like planting is similarly difficult to modify or improve due to the fairly simplistic nature. Biomass that has been bottom planted must be manually cut from the sea floor although machines are now available that are capable of cutting seaweed at low depths [156]. Long line offshore cultivation lines need to be collected with the use of a fishing boat or similar vessel.

The energy recovery techniques with macroalgae are limited by their composition. A low oil content makes the extraction of the oil likely to be unviable. The high moisture and high ash content mean thermochemical processes are also disadvantaged. The two promising recovery methods are fermentation/distillation to bioethanol and anaerobic digestion to methane. Due to recent discoveries related to the production of enzymes which can convert certain carbohydrate properties in

macroalgal biomass to fermentable sugars, the production of bioethanol from macroalgae appears promising. The research so far has only considered a limited number of species and alternative species which are capable of large scale cultivation should be considered. Anaerobic digestion provides an alternative method with lower energy consumption and potentially high yields of methane recovery [120, 124, 157]. The yields however are highly dependent upon the capacity of the species to be biodegraded. Potentially a combination of bioethanol production and subsequent anaerobic digestion of the residual waste could produce the greatest recovery of energy for the least input.

2.8 Conclusions

Clearly despite much research being conducted to bring about the commercialisation of energy recovery from algal biomass there are still major limitations hindering the concept. The limitations for microalgal and macroalgal biomass are very different. For microalgae the energy input remains too high in comparison to the potential output. The inputs to each of the processes need to be minimised where possible. Cultivation should be carried out in raceway ponds without plastic lining and only in areas where a suitable and readily available supply of wastewater is available. CO_2 injection should be conducted where there is a supply of flue gas, potentially from an electricity generating plant. The ponds should be seeded with a local species of algae that has been identified as being compatible with low energy harvesting and high energy recovery if possible. The preferred method of low energy harvesting should be filtration providing the species is large enough to be captured. Otherwise flocculation with an organic flocculant followed by sedimentation is recommended. Where necessary, centrifugation can be used to highly concentrate the biomass. Recovery of biodiesel is practical for species of algae that contain a high proportion of oil, however these specific species are difficult to cultivation especially in raceway ponds. It may be that research has taken the wrong path given that biodiesel recovery has received the vast majority of attention. Direct combustion of microalgae is a comparatively simpler process that can yield reasonable energy recovery through generation of electricity. The concept however relies upon the cheap and effective drying of biomass. Production of bioethanol has shown promise with high yields of

ethanol having been produced from a variety of strain of microalgae. Given the comparatively higher content of carbohydrates to oils in most species of algae it may provide a better recovery method over oil extraction for naturally occurring species. The energy demands of bioethanol production on a large scale are however relatively unknown and likely to be high, which could reduce the overall viability. Anaerobic digestion perhaps provides the best recovery method as the material and energy inputs are minimal with potentially high yields of methane recovery. Despite being a relatively low value fuel (in 2013 the value was calculated to be 0.003 $/MJ of methane compared to 0.019 $/MJ for bioethanol using values from the US Energy Information Administration [158] and Iowa State University [159] for natural gas and bioethanol respectively), methane is flexible in that it can be used to generate electricity which can power system processes and generate surplus electricity or upgraded to a transport fuel.

As with microalgae, the method of cultivation of macroalgae for bioenergy is largely location dependent. The species can be selected, however native species should be preferred due to their tolerance to local conditions. The method of cultivation depends upon the chosen species of algae with studies suggesting that long-line cultivation is favourable for productivity rates, lack of labour input and low biomass losses. Production of bioethanol from macroalgae has shown promising results especially for brown algae and if high yields are possible with many species of macroalgae using new techniques, the concept has great potential. Methane production provides a simple conversion of biomass to energy although yields differ greatly between species. Similarly to bioethanol, brown algae has been shown to produce high yields of methane and should be favoured. Supposing bioethanol recovery is possible, the residual waste from the process can be used for methane production to maximise the energy recovery.

3 Algal cultivation and conversion to bioenergy

3.1 Introduction

The purpose of this chapter is to investigate the potential for cultivating locally obtained species of algae and converting the biomass to biofuel and other products. There have been many studies conducted considering the cultivation of algae and its conversion to bioenergy but most have focussed upon specific species of algae for their particular properties [11, 30, 90]. Some species of algae have characteristics that make them particularly beneficial for cultivation and processing to bioenergy, for example, a high growth rate [30] or a high fraction of oil [30, 90]. The cultivation of specific species of algae in a controlled environment is relatively straightforward [58] however when cultivated in an open environment, contamination from other species often occurs [14, 34, 47]. Large scale cultivation of algae favours the use of raceway ponds due to simplicity and lower inputs [32, 37]. Ponds however, are generally open to the environment which means localised species are most likely to dominate [34]. For this reason the development of local species is potentially more beneficial than attempting to cultivate specific species. As opposed to examining the cultivation and processing of selected species this study tests local species of algae for its ability to be cultivated in open containers and converted to bioethanol, pyrolysis gases and biochar.

3.1.1 Cultivation of algal biomass in agricultural effluent

One of the major bottlenecks in the production of bioenergy from algal biomass is the high energy requirement of cultivation and the environmental impact associated with using fertilisers to provide the required nutrients and minerals for high productivity [27, 35]. Wastewater is an alternative source of nutrients [136], the use of which can have the dual benefit of aiding the growth of biomass while improving the quality of the effluent by removing a proportion of the nitrogen and phosphorous loading [141]. One type of wastewater with very high concentrations of nitrogen and phosphorous is swine effluent [160, 161]. Swine effluent is generally used as an agricultural fertiliser. With a great increase in the number of pig farms in parts of the world there has been a corresponding rise in effluent requiring treatment [162].

Traditionally it was common to use the effluent from pig farms for fertiliser on arable land. However with the designation of Nitrate Vulnerable Zones (NVZs) to control nitrate contamination of ground-waters and eutrophication [98, 101] the demand compared to supply has reduced, particularly in Europe [162]. As a result, effluent often requires transportation to areas where it can be used as fertiliser or to be treated to remove the high nitrogen content. Both of these measures can be expensive [162]. The high nitrogen and phosphorous content of swine effluent makes it an ideal candidate for algal cultivation with the algae utilising the nutrients for growth and providing removal. Cultivation of algae in swine effluent has been conducted previously using open ponds [160, 161], algal turf scrubbers [163] and photo-bioreactors [164]. Most of these studies recorded a high uptake of nitrogen and phosphorous in the effluent. Table 3-1 displays the method of cultivation, the species, hydraulic retention time (HRT) and the N and P removal rates for the forms measured.

Table 3-1 Comparison of nitrogen and phosphorous removal rates for different studies

Effluent	**Cultivation method**	**Algal species**	**Hydraulic retention time (days)**	**N removal (%)**	**P removal (%)**	**Ref.**
Pig slurry (Diluted 1:9)	Pilot scale raceway pond	*Chlorella vulgaris*	4.4-12.8	54-98	42-89	[161]
Pig farm wastewater (partially treated)	Lab scale pond	*Chlorella vulgaris*	3.8	79.2[1]	74.0[2]	[160]
Flushed swine manure effluent	Algal turf scrubber	Filamentous green algae consortia	-	>90	68-76	[163]
Pre-treated piggery effluent	Photo-bioreactor	*Chlorella sorokiniana*	7	94-100	70-90	[164]

(Note: [1]Inorganic N [2]Orthophosphate)

Each of the studies in the table recorded a fairly high removal of nitrogen and phosphorous from the effluent used. Three of the studies used *Chlorella* sp. as the algae for nutrient removal. Being a microalgae however, *Chlorella* species are difficult to harvest and generally require flocculation or centrifugation [58].

Filamentous algae, like the species used in this study have a larger cell size providing a better biomass in terms of harvesting potential as they can be easily filtered [83]. They have been less researched, however, because they generally contain a lower fraction of oils than microalgae [165]. There is potential due to their high polysaccharide content for conversion to bioethanol [149].

3.1.2 Conversion of biomass to bioethanol

As a result of the high oil content of some algal species and the high value of biodiesel, most studies investigating biofuel production from algae concentrate on biodiesel production. While this makes sense for selected species with a high lipid content [36], the oil content of naturally dominant species often tends to be low [141]. A typical range of lipid contents for wild species is from 1.5 to 10.5% [166] with the suggestion that algae with a lipid content below 40% is not worth considering for the production of biodiesel [36]. As mentioned previously it is generally understood that large scale cultivation of algae can only be achieved through the use of large ponds due to the lower energy inputs in comparison to alternative methods, notably the use of photobioreactors [37]. One of the major drawbacks of the use of ponds however is the contamination of the ponds by local species of algae [47]. It is therefore necessary to operate a biomass processing stream that is applicable to naturally dominant species of algae. Often, filamentous algal species are the dominant species in the natural environment [167] which tend to have a higher polysaccharide content compared to unicellular species and are more suited to the production of bioethanol [168] than biodiesel. The absence of lignin in algal biomass [169] also suggests that high conversion efficiencies may be possible as the presence of lignin is often an obstacle to bioethanol production [169].

The production of bioethanol from algae has received little attention in comparison to biodiesel production, however, positive results have been recorded [99, 101, 170, 171]. Table 3-2 displays bioethanol recovery rates that have been obtained from algal biomass from different studies and the conditions/materials used. Bioethanol recovery rates from other common bioethanol feedstocks have been included for comparison.

Table 3-2 Bioethanol recovery rates from different species of algae and from conventional feedstocks with the conditions used

Species	Pre-treatment	Yeast	Ethanol Recovery rate (%)	Refs.
Chlorococum sp.	Supercritical lipid extraction	*Saccharomyces bayanus*	38	[100]
Chlorella vulgaris	Sulphuric acid	*Zymomonas mobilis*	23.3	[149]
Chlamydomonas reinhardtii	Sulphuric acid	*Saccharomyces cerevisiae*	29.2	[101]
Chlamydomonas reinhardtii	Enzymatic	*Saccharomyces cerevisiae*	23.5	[172]
Arthrospira (*Spirulina*) *platensis*	None	*Saccharomyces cerevisiae*	35	[173]
Spirogyra sp.	Enzymatic	*Saccharomyces cerevisiae*	8	[174]
Sugar cane bagasse	Ammonium hydroxide	*Saccharomyces cerevisiae/Pichia stipitis*	48	[175]
Sorghum	Ammonium hydroxide	*Saccharomyces cerevisiae*	11.6-13.0	[176]
Corn stover	Hydrothermal and enzymatic	*Escherichia coli*	27	[177]

Research conducted investigating bioethanol recovery from various algal strains suggests that the feedstock is capable of producing ethanol at a recovery rate similar to conventional feedstocks. The results displayed in table 3-2 show that some species are capable of providing an ethanol yield greater than that recorded from corn stover which is considered a promising feedstock [177]. The study that investigated bioethanol production from *Spirogyra* sp. [174] recorded low yields of ethanol in comparison to the other species. Promising results were recorded by another study for the filamentous cyanobacteria *Spirulina platensis* [173]. Due to the current lack of data it is difficult to draw strong conclusions regarding the favoured species, particular characteristics and pre-treatment methods for effective bioethanol production from algal biomass. Nevertheless, the research that has been conducted shows that several species of algae can produce high yields of bioethanol that could

compete with bioethanol produced from more conventional feedstocks such as sugar cane, sorghum and corn stover.

This work investigated the potential bioethanol conversion from locally collected algae, identified as being mostly *Spirogyra* sp by a geneticist within the University of Edinburgh. This species of freshwater algae is one that is commonly found all over the world in ponds and rivers [178, 179] and is therefore a species that could be cultivated in many locations without the risk of contamination. The biomass was collected and dried before being ground, enzymatically hydrolysed and fermented to bioethanol using yeast. Alternative feedstocks were also tested for comparison which included coppiced willow, synthetic municipal solid waste, seaweed (*Fucus vesiculosis*). α-cellulose was also hydrolysed and fermented to test the effectiveness of the method used. The glucose concentrations obtained from each biomass were measured during the hydrolysis and the ethanol concentrations were measured kinetically during the fermentation process.

3.1.3 Pyrolysis of biomass to biochar, bio-oil and syngas

Aside from bioethanol production, the process of pyrolysis was also investigated as a method of recovering energy from the biomass. Pyrolysis is the thermal treatment of biomass under oxygen limited conditions which produces biochar, bio-oil and syngas. The process of pyrolysis has been identified as an effective method of carbon sequestration by converting biomass to biochar. During photosynthesis the biomass uptakes atmospheric CO_2 and after pyrolysis the biochar can be applied to soil where the carbon is then effectively locked for long periods of time [180]. Biochar has also been proven to be an effective soil amender, improving the characteristics of soil and increasing crop productivity [181]. Aside from providing a method of carbon sequestration and producing biochar as a soil amender, the process of pyrolysis also produces bio-oil and syngas, both of which can be used as energy carriers [182]. Bio-oil has highly complex characteristics and unrefined bio-oil cannot be used directly as a fuel due to its instability and high acidity. Upgrading of the oil, however, can allow it to be used as a transport fuel [183]. Syngas is composed of a number of different gases, mainly hydrogen, carbon monoxide, carbon dioxide, methane and ethylene [184]. The energy contained within the gas means that the gas can be used

directly in a gas turbine [182] or the gas can be upgraded to a more valuable product [184]. The product proportions can be adjusted by varying the reaction time and temperature of the process. In general, the higher the temperature of the pyrolysis the greater the recovery of the energy products (syngas and bio-oil) and conversely when the reaction temperature is lower, a higher proportion of biochar is produced [182].

Algal biomass has been considered as a potential feedstock for pyrolysis as a method of carbon sequestration and bioenergy generation [185]. The high productivity of algal biomass in comparison to terrestrial crops leads to a higher potential for carbon sequestration and energy recovery [185]. The cultivation and processing of algal biomass to bioenergy has been calculated as being energetically sustainable in some studies [10, 35]. Most studies, however, generally consider the processing of the biomass to biodiesel or biogas [10, 27, 33, 35, 37]. Biodiesel is commonly researched due to the high oil contents of some species of algae [186], however the cultivation and processing of the biomass is energy intensive and it is suggested that under only certain conditions the concept is viable [35]. Lardon *et al.* [35] found that under normal conditions of cultivation, just the drying of the biomass for processing consumed 87% of the energy produced. This is without including the energy consumed through cultivation, oil extraction and transesterification, when this was included the energy balance was -2.6 MJ for every 1 kg of biodiesel produced. The processing of the biomass to biogas is attractive due to the lower processing intensity however biogas is not a particularly high value bioenergy carrier. In comparison to both of these processing methods, aside from bioenergy recovery, pyrolysis has the benefit of carbon sequestration as a fraction of the biomass ends up being retained in soil following application. Chaiwong *et al.* [185] found that the bio-oil produced from the pyrolysis of *Spirulina* Sp. had a higher heating value greater than that of bio-oil from wood and the system had a net positive energy gain. Hu *et al.* [187] pyrolysed *Chlorella vulgaris* with the aim of maximising bioenergy generation from the syngas produced. The authors found that the fast pyrolysis of the biomass at 800°C provided the best conditions for energy recovery. Babich *et al.* [188] focussed upon the recovery of bio-oil from *Chlorella* Sp. Where 28% bio-oil was recovered from the biomass which for a heating value of 33 MJ/kg corresponded to an energy recovery of 42%. Current research suggests that algal biomass can provide a good

feedstock for bioenergy recovery and carbon sequestration via pyrolysis although the number of species and conditions tested remain limited.

This experiment investigates the products produced by the pyrolysis of the locally obtained freshwater algal biomass. The pyrolysis of synthetically produced municipal solid waste was also conducted to provide a comparison of the results. The purpose was to assess whether this process could provide a useful method for energy recovery from algal biomass.

3.2 Materials and methods

3.2.1 Cultivation of algae in agricultural effluent

Swine effluent was collected from a pig farm in the south-west of Scotland. The effluent was taken from an effluent holding tank. Samples of the effluent were added to volumetric flasks and diluted with de-ionised water to produce effluent of different concentrations: 1:20, 1:50 and 1:100 (effluent: DI water). The effluent samples were then placed in autoclavable flasks and autoclaved at 121°C for 15 minutes to remove pathogens. 150 ml of each different dilution was poured into four open plastic rectangular containers. The containers were placed in the laboratory beside a window facing approximately north-east (See Fig. 3-1). A 36 W fluorescent lamp was centred 15 cm above the containers on a light/dark period of 12h/12h to provide an extra source of light. At the start of the experiment the ammonium and phosphate concentrations were measured using spectrophotometric test kits (Spectroquant). For ammonium measurement, 0.2 ml samples from each of the containers were taken and diluted with 9.8 ml of de-ionised water in a 10 ml volumetric flask. The samples were then reacted with reagents from the ammonium test kit. Reacted samples were poured into a spectrophotometer cell and the absorbance measured in the spectrophotometer (Thermo Scientific Helios Alpha) at a wavelength of 650 nm. Using a calibration curve produced with ammonium phosphate (R^2 = 0.9996) (see Appendix A-1), the concentration of ammonium was calculated for each sample based on the absorbance of each sample. For the measurement of phosphate another sample (1 ml) was taken from the containers and diluted with 9 ml of de-ionised water. The samples were reacted with reagents from the phosphate test kit. The samples were placed into a spectrophotometer cell and the absorbance values were

measured at a wavelength of 480 nm. Using a calibration curve produced with potassium phosphate (R^2 = 0.9995) (see Appendix A-1) the PO_4 concentration values of the samples were calculated based on the values of absorbance.

Samples of freshwater algae were obtained from a pond in south-west Scotland, the algae was identified as being predominantly *Spirogyra sp.* Fig. 3-2 shows a microscopic photograph of the algal biomass. After the ammonium and phosphate concentrations of the effluent in the containers were determined, nine 0.15 g (w.w.) samples of locally obtained freshwater algae (*Spirogyra sp.*) were weighed on an analytical balance and added to three containers of each dilution leaving one container per dilution as a blank (See Figure 3-3).

Figure 3-1 Set up of algal cultivation in diluted swine effluent

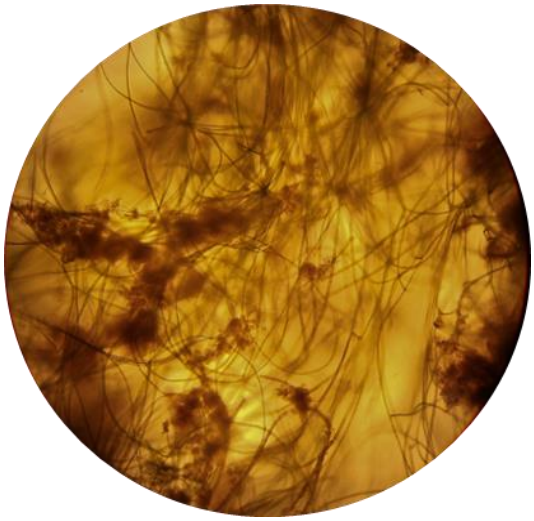


Figure 3-2 Microscopic photograph of freshwater algae obtained for the cultivation experiment

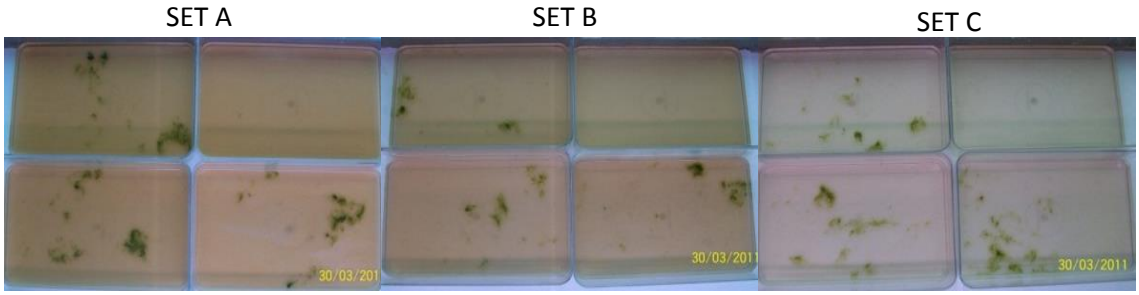


Figure 3-3 Algal biomass placed in open containers containing swine effluent with different dilution ratios (SET A - 1:20, SET B - 1:50, SET C - 1:100)
(Note: In each set the top right container is blank)

The same procedure for measuring ammonium and phosphate concentrations was conducted every two days to measure the decrease in the concentrations. The dilution of the samples varied to allow the concentration to be within the range detectable by the colorimetric method used. The evaporation in the containers was measured each day by measuring the volume lost in the blank container. De-ionised water was added to the containers to replace the evaporated water. The daily evaporation ranged from 19 to 27 ml per container. After eight days of growth the algal biomass was removed with tweezers (Fig. 3-4) and weighed using an analytical balance, this gave the mass (w.w.) of the biomass. Nine aluminium foil sheets were then cut to use as containers for drying the algae on. Each sheet was marked and the mass measured using an analytical balance. The samples of biomass from each container were then placed on separate sheets and dried at 105°C overnight. The dry biomass and foil sheet was then weighed with an analytical balance, the dry mass minus the mass of the sheets gave the dry mass of the algal biomass (Appendix A-1).

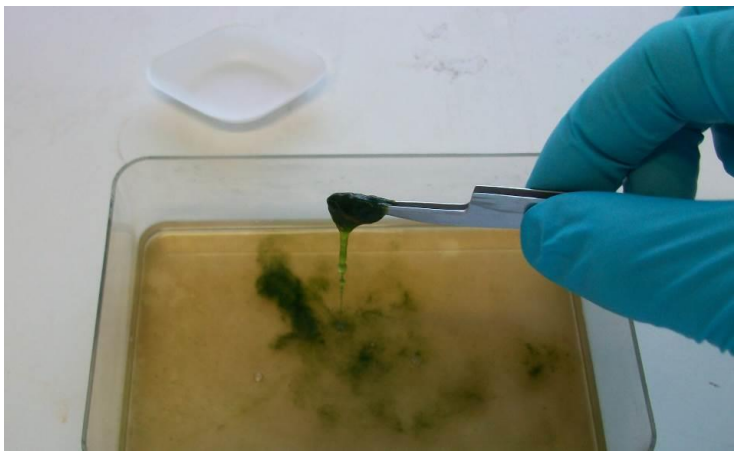


Figure 3-4 Removal of algal biomass from container using tweezers

3.2.2 Outdoor cultivation of algal biomass with nutrient addition

Cultivation of the local algal species used above was also conducted in containers outdoors using pond water with added nutrients after finding this an effective medium. Several different media types had been tested in the laboratory using Winchester bottles (see Appendix A-1). The sterilised pond water enriched with nitrogen and phosphorous appeared to provide the best medium for growth of the wild algae. To test the best concentration of nitrogen and phosphorous, 5 plastic containers were set up outside containing sterilised pond water with 0, 20, 50 80 and 100 mg/L of nitrogen using NH_4NO_3 and 0, 2.9, 7.4, 11.1, 14.3 mg/L of phosphorous using KPO_3. 20 g (w.w.) of wild algal biomass was added to each of the containers and the growth was monitored over a period of 16 days. Typical values of nitrogen in growth medium are 124 mg N/L and 5.3 mg P/L (3N Bold Basal Medium) or 247 mg N/L and 0.71 mg/L [189]. Figure 3-5 displays the set-up of the experiment and the nitrogen and phosphorous concentrations added to each container.

1	2	3	4	5
N = 0 mg/L P = 0 mg/L	N = 20 mg/L P = 2.9 mg/L	N = 50 mg/L P = 7.4 mg/L	N = 80 mg/L P = 11.1 mg/L	N = 100 mg/L P = 14.3 mg/L

Figure 3-5 The set-up for the cultivation of wild algal biomass in sterilised pond water with varying concentrations of nitrogen and phosphorous

3.2.3 Indoor cultivation of pure algal biomass species

A pure strain of *Spirogyra* was obtained from the Scottish Association for Marine Science and was cultivated in the lab to test the ability of the species to grow without contamination issues. A medium of Basal Bold with three fold nitrogen (124 mg N/L) and vitamins was prepared and sterilised. 50 ml of the medium was added to six Erlenmeyer flasks. 1 ml of the species stock was then added to each flask and the flasks were placed on a shelf in front of a westward facing window beneath fluorescent lighting on a 12:12 hour cycle. Photographs of the flasks were taken regularly to allow observational measurement of the growth.

3.2.4 Conversion of biomass to bioethanol

Several biomass types were tested for their capacity for conversion to bioethanol including a species of freshwater algal biomass collected locally. The algal biomass used for this experiment was collected from a local pond in Edinburgh in August 2011, the biomass was observed to be mainly *Spirogyra* sp. Coppiced willow was taken from an area within the King's Buildings campus of the University of Edinburgh. The municipal solid waste was made up in the laboratory and was based on typical materials found in kitchen and garden waste. The municipal solid waste was made up of food waste (carrot peel, onion skin, potato peel, lettuce leaves, tomato skin and cucumber skin, 16.7% of each) (16%), straw (7%), grass cuttings (37%), sawdust (21%) and leaves (19%). Seaweed was collected from Cramond beach, Edinburgh and was identified as *Fucus vesiculosis*. The α-cellulose powder

was purchased from Sigma Aldrich. All of the biomass was dried in a furnace at 105°C overnight and the following day was ground with a pestle and mortar before being sieved through a 0.2 mm sieve. Duplicate 0.5 g samples of the biomass were then weighed out on an analytical balance and poured into 250 ml Erlenmeyer flasks. 45.9 ml of de-ionised water was then added to each flask and aluminium foil was placed on the top and secured with autoclave tape. Each of the flasks were then weighed. The flasks were autoclaved at 121°C for 15 minutes. Once the flasks had cooled to room temperature the masses of the flasks were measured and the mass lost was recovered by adding the corresponding volume of autoclaved de-ionised water. Enzymes were then added to the broth to facilitate the conversion of polysaccharides to simple sugars. 0.1 ml of cellulase complex (Novozymes NS50013) mixed with 0.9 ml de-ionised water was added alongside 0.06 ml β-glucosidase (Novozymes NS50010) and 0.04 ml enzyme complex mix (Novozymes NS50012). To keep the pH of the broth at 4.8 (the oprimum pH for enzymatic hydrolysis), 2.5 ml of citrate buffer was added to the flasks. The total volume of each flask was then 50 ml containing 0.5 g of biomass. The flasks were then placed on an orbital shaker at 250 rpm in an incubator at 50°C. The glucose concentrations were measured after 24 hours and then again at 48 hours using a glucose meter (Accu-Chek). After 48 hours of hydrolysis, 0.05 g of FALI yeast (*Saccharomyces cerevisiae*) (supplied by AB Mauri) dissolved in 0.5 ml of de-ionised water was added to each flask aseptically. The flasks were returned to the incubator and placed on the shaking table at 250 rpm at 30°C. At 3, 6, 12 and 24 hour intervals, 0.2 ml samples from each of the flasks were extracted and diluted with 1.8 ml of de-ionised water containing 1 g/L methanol. The samples were then capped and run through a gas chromatograph with flame-ionisation detector (GC-FID) to detect the ethanol and methanol concentrations (See Appendix A-2 for details of the method used). The R ratio was determined for each sample (the ratio of ethanol to methanol) and using a calibration curve ($R^2 = 0.9994$) (Appendix A-2), the ethanol concentrations were calculated.

3.2.5 Pyrolysis of locally obtained algal biomass and municipal solid waste

The freshwater algal biomass was the same used for bioethanol production which was obtained locally from a pond in Edinburgh and observed to be mainly *Spirogyra* sp. The municipal solid waste was made up in the laboratory using food waste (carrot peel, onion skin, potato peel, lettuce leaves, tomato skin and cucumber skin, 16.7% of each) (16%), straw (7%), grass cuttings (37%), sawdust (21%) and leaves (19%). The moisture content of the biomass was measured gravimetrically by drying the biomass overnight at 105°C and measuring the loss in mass. After being dried, the samples were stored in a desiccator. For each pyrolysis run a proportion of the dried biomass was weighed and inserted into the pyrolysis set up.

The pyrolysis was conducted using the apparatus shown in Fig. 3-6. The set-up consisted of a static bed reactor which was a vertical 50 mm diameter quartz tube with a sintered plate at the base. The samples were placed in the quartz tube at a depth of about 200 mm. The samples were then heated by a 12 kW infrared gold image furnace (P610C; ULVAC-RIKO, Yokohama, Japan) with a proportional–integral–derivative (PID) controller. The temperature of the sample bed was monitored and controlled by a thermocouple that was positioned 10 mm from the inner surface of the quartz tube. A pipe supplying nitrogen gas (N_2) was attached to the bottom of the pyrolysis tube and nitrogen was passed through the tube at a controlled rate. As the reactor was heated, the gas passed up through the sample removing volatiles and the syngas into a condensation system consisting of two sections. The first section was heated at 160±10 °C. This section removed entrained particulates on a filter and collected high-boiling tars in a separate trap. The second section consisted of a series of condensers and receivers where further condensable liquid products were collected. Data for the main process variables, temperature, pressure and gas volume flow were logged in real time.

The biomass was added to the quartz tube before the whole system was assembled and both biomass samples were run in duplicate. The mass of the sample tube was measured, the dry biomass was added to the tube and the final mass measured. The pressure sensors were zeroed and the system was purged with nitrogen gas before

establishing a steady nitrogen gas flow rate of 0.33 l/min as carrier gas (which gave a linear cold flow velocity within the empty pyrolysis tube of approximately 3 mm/s). Samples from all feedstock types were heated at a rate of 20 °C/min. A hold temperature of 600 °C was used and maintained for 20 min before gradual cooling (with continued nitrogen gas flow) until below 100 °C.

Following the pyrolysis, the product masses were determined for the char and condensed liquids by weighing the equipment containing the products and subtracting the masses measured before the experiment. The product gas volume was measured using a volumetric flow meter (TG5; Ritter, Bochum, Germany). The composition of the gas was measured using a mass spectrometer (HPR-20 QIC; Hiden Analytical Ltd, Warrington, UK). The masses of each gas were determined by calculation using the proportion of each gas and the total volume of gas produced.

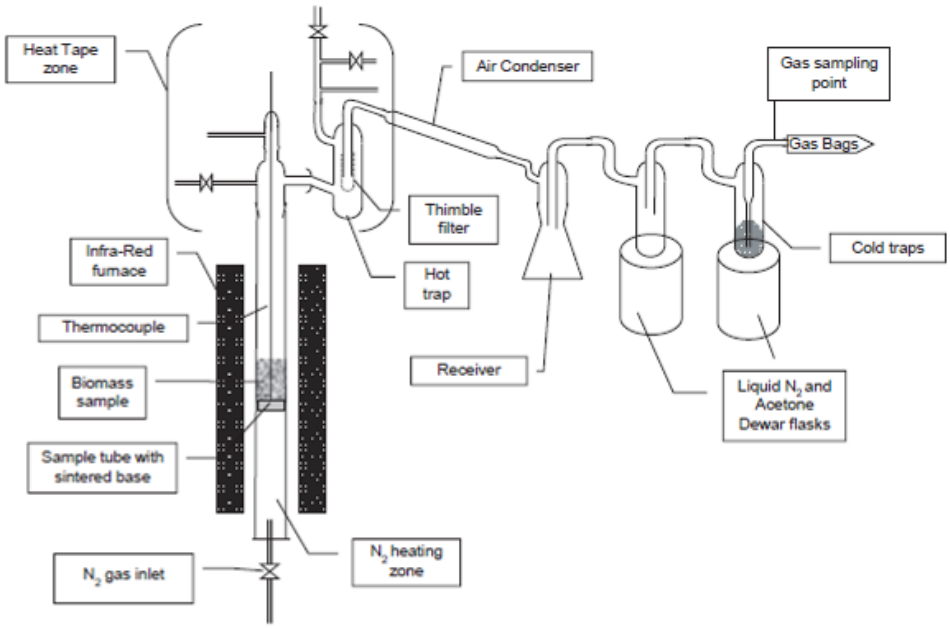


Figure 3-6 Set-up of the small-scale pyrolysis unit, UKBRC, The University of Edinburgh (Note: Diagram obtained from Crombie *et al.*[190])

3.3 Results and discussion

3.3.1 Cultivation of algae in agricultural effluent

3.3.1.1 Nutrient removal

A local species of freshwater algae was collected and cultivated in agricultural effluent to test the productivity and removal of nutrients in the effluent. The concentrations of NH_4^+ and PO_4^{3-} were measured at the start of the experiment, the mean initial concentrations of each different dilution are displayed in Table 3-3.

Table 3-3 Initial concentrations of NH_4 and PO_4 for each dilution (standard deviation in parentheses)

Set	NH_4^+ conc. (mg/L)	PO_4^{3-} conc. (mg/L)
A	23.02 (0.26)	41.45 (0.55)
B	13.94 (1.08)	27.87 (0.86)
C	8.08 (0.68)	15.95 (3.27)

From inoculation, samples were taken every two days to measure the ammonium and phosphate concentrations. Figure 3-7 displays the mean concentration of NH_4 for each set of initial concentrations over the total time. Figure 3-8 displays the concentrations of NH_4 normalised to the initial concentration for each set. Figures A-1 and A-2 in Appendix A display the concentrations of ammonium and phosphate of the blank containers.

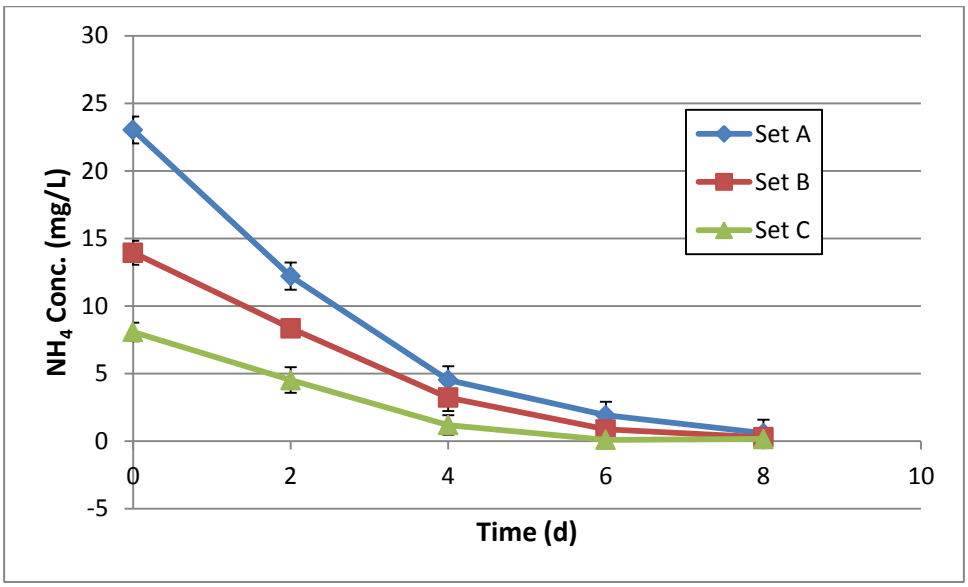


Figure 3-7 Ammonium concentration over time for each set (error bars displays standard deviation from triplicate samples)

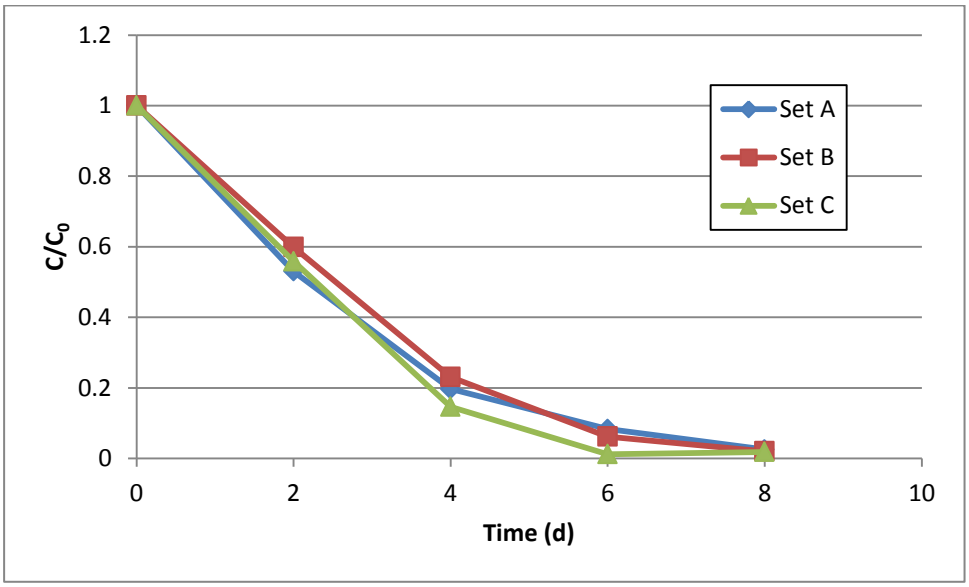


Figure 3-8 Ammonium concentrations normalised to initial concentrations over time for each set

The graphs show a clear reduction in ammonium concentrations for each set with table 3-4 displaying the initial concentrations of ammonium and the concentration measured after 8 days of growth with the percentage reduction.

Table 3-4 Concentrations of ammonium at day 0 and day 8 and the percentage reduction (standard deviation in parentheses)

	NH_4^+ conc. day 0 (mg/L)	NH_4^+ conc. day 8 (mg/L)	% reduction
A	23.03 (0.26)	0.58 (0.36)	97
B	13.90 (1.08)	0.28 (0.02)	98
C	8.08 (0.68)	0.15 (0.02)	98

Similarly to ammonium, samples were taken every two days to measure the phosphate concentrations. Figure 3-9 displays the mean concentration of PO_4^{3-} over time. Figure 3-10 displays the concentration of PO_4^{3-} normalised to the initial concentration over time. Table 3-6 displays the initial concentration of PO_4^{3-}, the final concentration and the percentage removal.

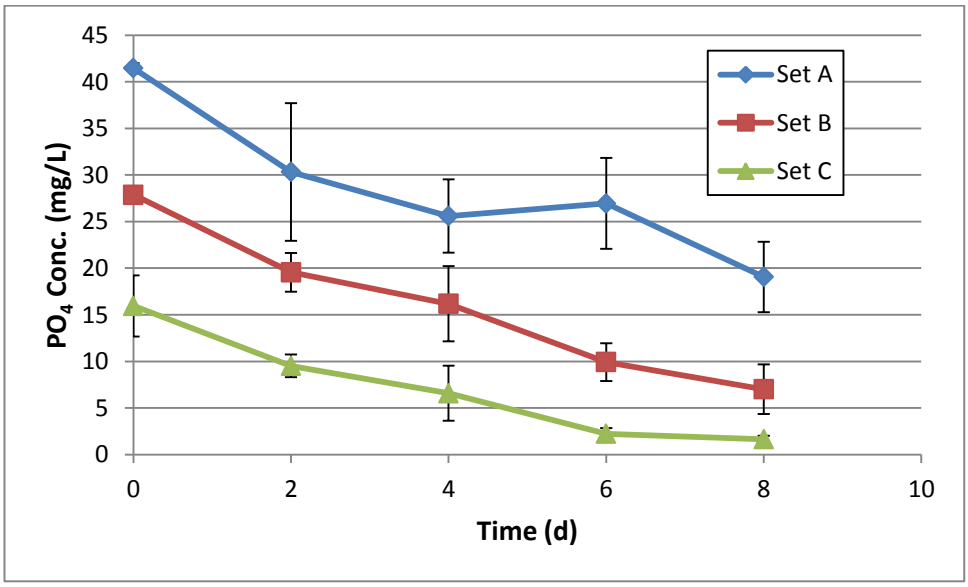


Figure 3-9 Phosphate concentrations over time for each set (error bars show standard deviation from triplicate samples)

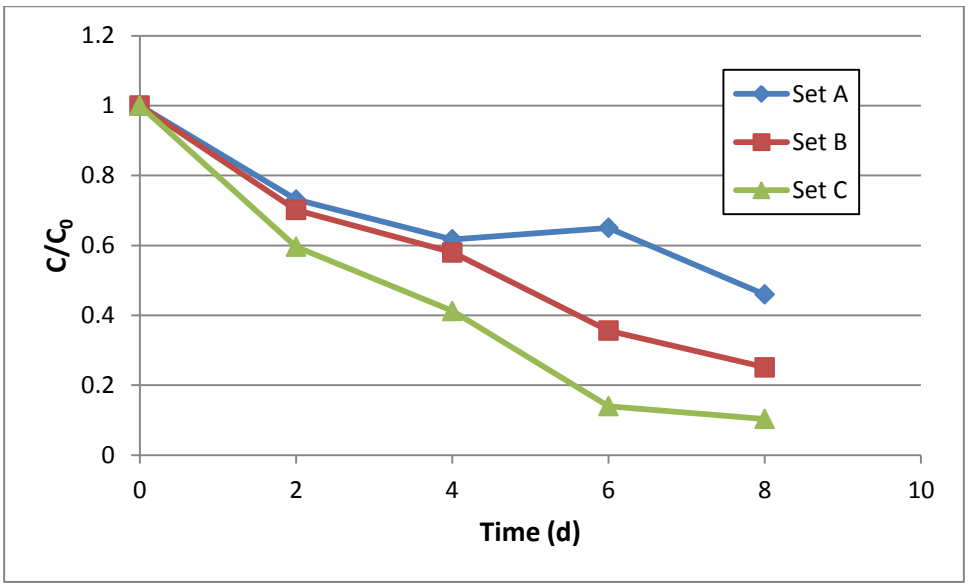


Figure 3-10 Phosphate concentrations normalised to the initial concentration over time for each set

Table 3-5 Concentrations of phosphate at day 0 and day 8 and the percentage reduction (standard deviation in parentheses)

	PO_4^{3-} conc. day 0 (mg/L)	PO_4^{3-} conc. day 8 (mg/L)	% reduction
A	41.45 (0.55)	19.06 (3.77)	54
B	27.87 (0.86)	7.01 (2.55)	75
C	15.95 (3.27)	1.65 (037)	90

For each set, the ammonium concentrations reduced rapidly. By day 4 the concentrations were well below half of the initial concentration with 85% reduction recorded for set C. After 8 days the ammonium concentrations were almost at 0 for each set. When the concentrations were normalised to the initial concentrations the rates of reduction for each set were very similar. At around day 4 the concentrations were almost equal for each set suggesting that in set A, with the greatest initial concentration, the algae were developing more quickly and therefore using a greater amount of ammonium. It should be noted however that in the blank containers the concentration of ammonium also reduced considerably although not to the same extent as the containers containing algal biomass. There may have been some bacteria developing in the containers which facilitated the reduction of ammonium in the effluent. Nevertheless, the results show a clear reduction in ammonium concentration to levels up to a removal of 98%.

The phosphate concentrations are observed to reduce in Figure 3-9 however at a slower rate than ammonium. The main reason for the comparatively low rate of reduction is because the initial concentrations of phosphate are higher than those of ammonium and thus the growth media becomes nitrogen limited before becoming phosphorous limited. Additionally freshwater green algae has a greater requirement for nitrogen over phosphorous according to the general molecular formula: $C_{106}H_{263}O_{110}N_{16}P$ [77]. The normalised concentrations in Figure 3-10 show a similar reduction in concentration for sets B and C however set A has a different reduction gradient. The concentration detected at day 6 is slightly higher than that of day 4 suggesting an error with this result or indeed the result for day 4. Similarly to ammonium, the phosphate concentrations also decreased in the blank containers which suggests that the phosphate reduction was not solely the result of the algal growth. Again, however, the decrease in the phosphate in the containers with the algae was greater than the blank containers and in set C the phosphate was reduced to 10% of the initial value.

The high removal rates were similar to the other studies that have considered algal biomass cultivation for removal of nitrogen and phosphorous in swine effluent. Kebede-Westhead *et al.* [163] and De Godos *et al.* [164] both recorded nitrogen uptake rates greater than 90%. In the studies, the phosphorous reduction was also lower than the nitrogen reduction and the reduction percentage was in the same range from 54-90%. The phosphorous uptake in the study by Fallowfied *et al.* [161] for example was between 42-89%.

The results indicate that the cultivation of the species used in this study provides a good removal of ammonium and phosphate from pig farm effluent. Given the characteristics of the species, it is easily harvested and can be used as a source of biomass for energy recovery of other added value products.

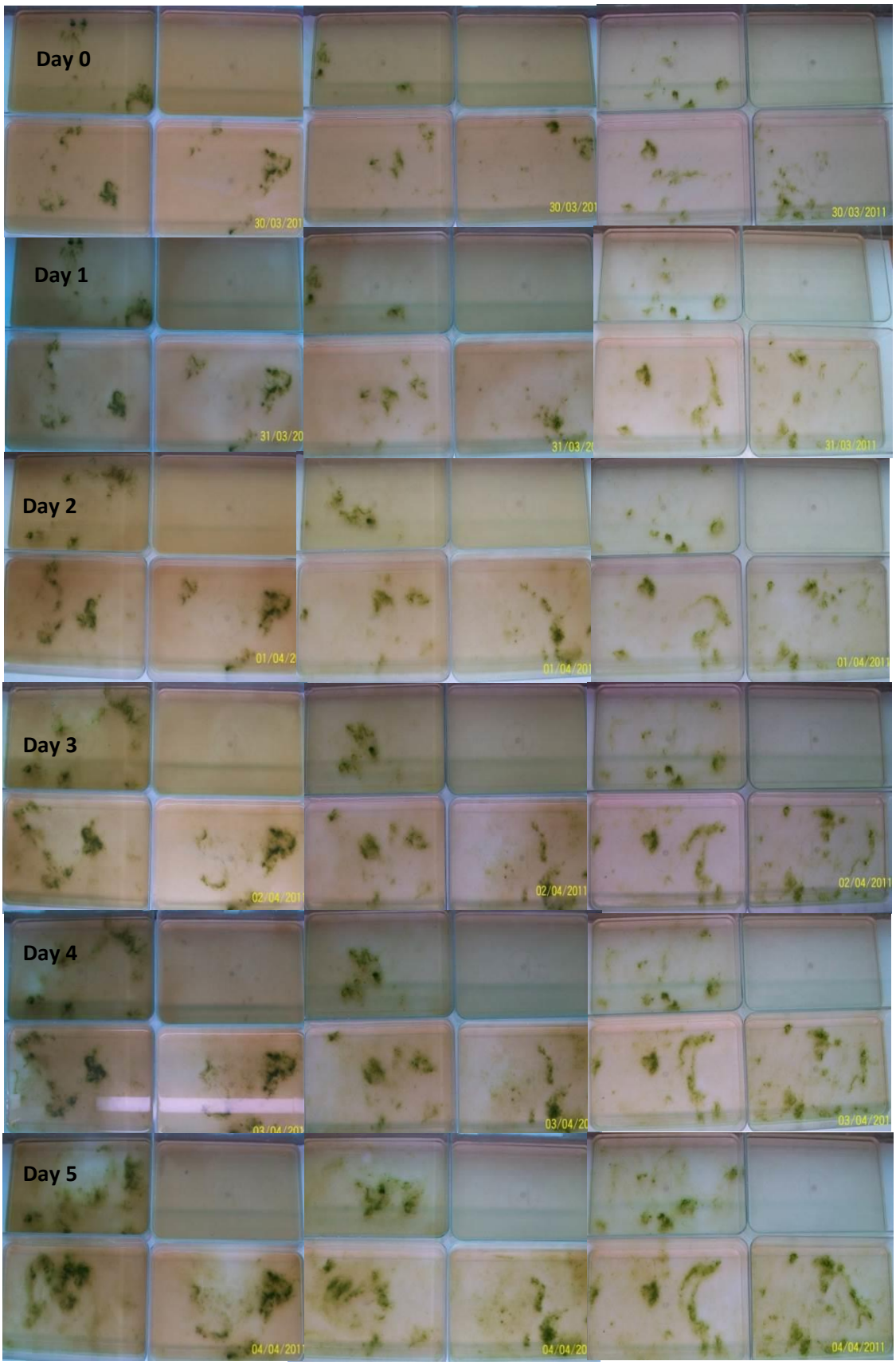
3.3.1.2 Biomass productivity

The productivity of the biomass was also measured during the experiment by determining the mass at the start of the experiment and again at the end. For each container 0.15 g samples of wet biomass were measured at the start of the experiment. The total solids content for the algae was determined to be 2.36 % ±

0.10 % (See Appendix A-1), the dry weight of each sample was therefore calculated to be 3.5 mg. At day 8 the mass of the wet biomass was measured by extracting the biomass with tweezers and placing the sample on an analytic balance. The samples were then dried and the mass of dry biomass measured. Using these measurements the productivity over the eight days was calculated. The results can be observed in Table 3-6. Photographs of the biomass in the containers at day 0 and day 8 can be viewed in Figure 3-11.

Table 3-6 Masses of biomass at the start and end of the 8 day experiment (standard deviation in parentheses)

	Day 0 (mg w.w.)	**Day 0 (mg d.w.)**	**Day 8 (mg w.w.)**	**Day 8 (mg d.w.)**	**Productivity (mg d.w./L/day)**	**Growth rate (/d)**
A	150	3.54	1,122 (96)	27.9 (1.7)	23.2 (1.4)	0.314
B	150	3.54	1,017 (221)	22.6 (5.4)	18.9 (4.5)	0.284
C	150	3.54	988 (202)	16.0 (3.7)	13.3 (3.1)	0.238


Set A
Set B
Set C
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5

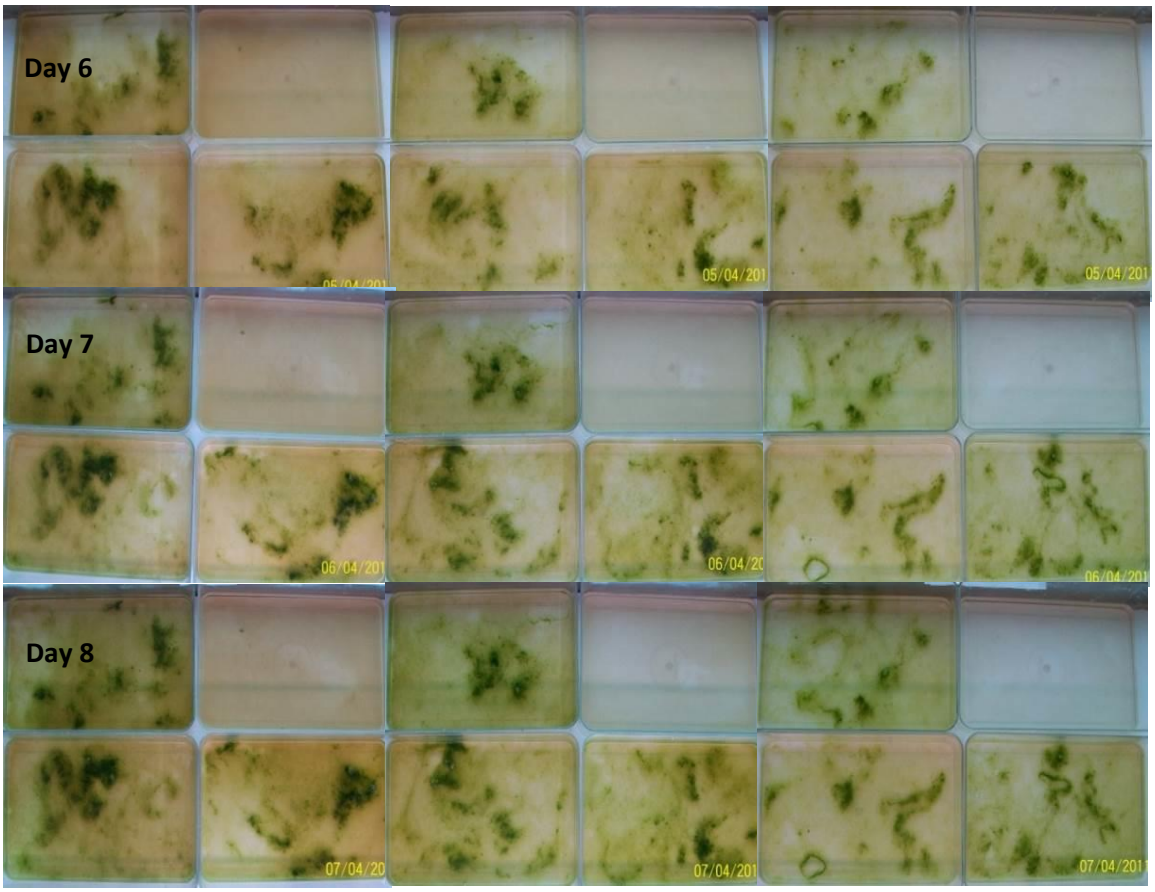


Figure 3-11 Photographs of biomass from day 0 to day 8 for each different set of effluent concentrations (A, B and C)

Given the productivity of the biomass, the theoretical N and P uptake values were calculated based on the general molecular formula of green algae ($C_{106}H_{263}O_{110}N_{16}P$) [77] and the corresponding mass of ammonium and phosphate calculated using stoichiometry (Table 3-7). This nutrient reduction calculation assumes that all of the nitrogen and phosphorous in the biomass is sourced from the NH_4^+ and PO_4^{3-} respectively. There will naturally be other sources of N and P in the effluent which will be used by the algae. Additionally the molecular formula used is an approximation of the ratios of elements in algae, for both of these reasons the results are therefore only an indication of the theoretical uptake of N and P.

Table 3-7 Theoretical NH_4 and PO_4 uptake based on the biomass productivity

	Initial NH_4^+-N conc. (mg/L)	**Initial PO_4^{3-}-P conc. (mg/L)**	**N uptake (mg/L/d)**	**Total N uptake (mg/L)**	**N uptake (%)**	**P uptake (mg/L/d)**	**Total P uptake (mg/L)**	**P uptake (%)**
A	22.63	13.52	1.47	11.73	51.83	0.62	4.98	36.83
B	13.66	9.09	1.19	9.52	69.70	0.50	4.04	44.44
C	7.94	5.20	0.841	6.72	84.63	0.43	3.40	65.38

The results from the experiment show that in set A the productivity was greatest and in set C it was lowest suggesting that the higher nutrient contents of set A benefited the growth of the algae. The relative abundance of nitrogen and phosphorous in set A will have allowed the biomass to continue developing before nutrient limitation occurred. The results suggest at these levels of dilution the higher the concentration of nutrients, the higher the productivity. The high concentrations of nutrients in the diluted effluent didn't appear to have any adverse impacts upon the growth. The effluent in set A was also clearly more turbid which could have led to lower productivity rates by causing photo-inhibition but little impact was observed. There has been little research investigating the productivity of *Spirogyra* sp. One study that specifically looked at the cultivation of *Spirogyra* sp., [191] measured a growth rate of 0.224 /d which is slightly lower than the growth rate measured in this study. Despite this positive comparison, relative to the productivities of many species of microalgae, the productivity of the species measured in this study is low. The maximum productivity measured in this study was 23.3 mg/L/day. For many species of microalgae the productivity is a magnitude higher, for example [30] measured productivities of 0.28 g/L/day, 0.26 g/L/day and 0.23 g/L/day for *Chlorococcum* sp., *Scenedesmus* sp. and *Chlorella Sorokiniana* respectively. It should be noted however that these productivities were obtained under intensive conditions in flasks on an orbital shaker with CO_2 sparging.

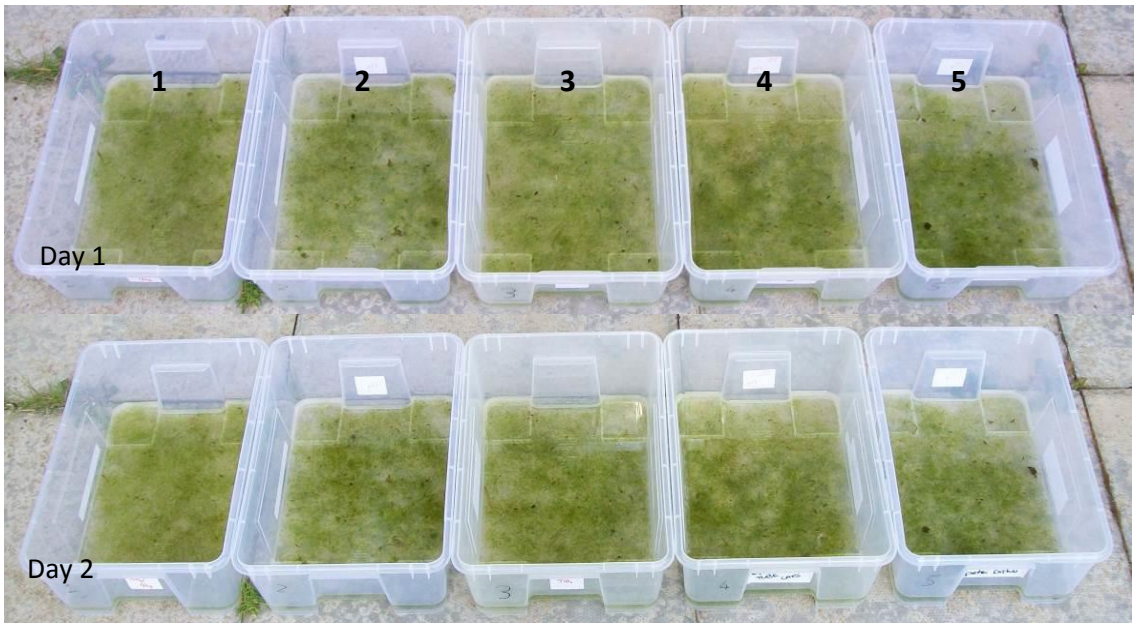
The theoretical uptake of N and P did not correlate particularly well with the colorimetric measurements of the NH_4^+ and PO_4^{3-} uptake. According to the theoretical uptake values, less N was taken up than was calculated by colorimetric measurements for all of the sets. A maximum N uptake was calculated as 84.6% in

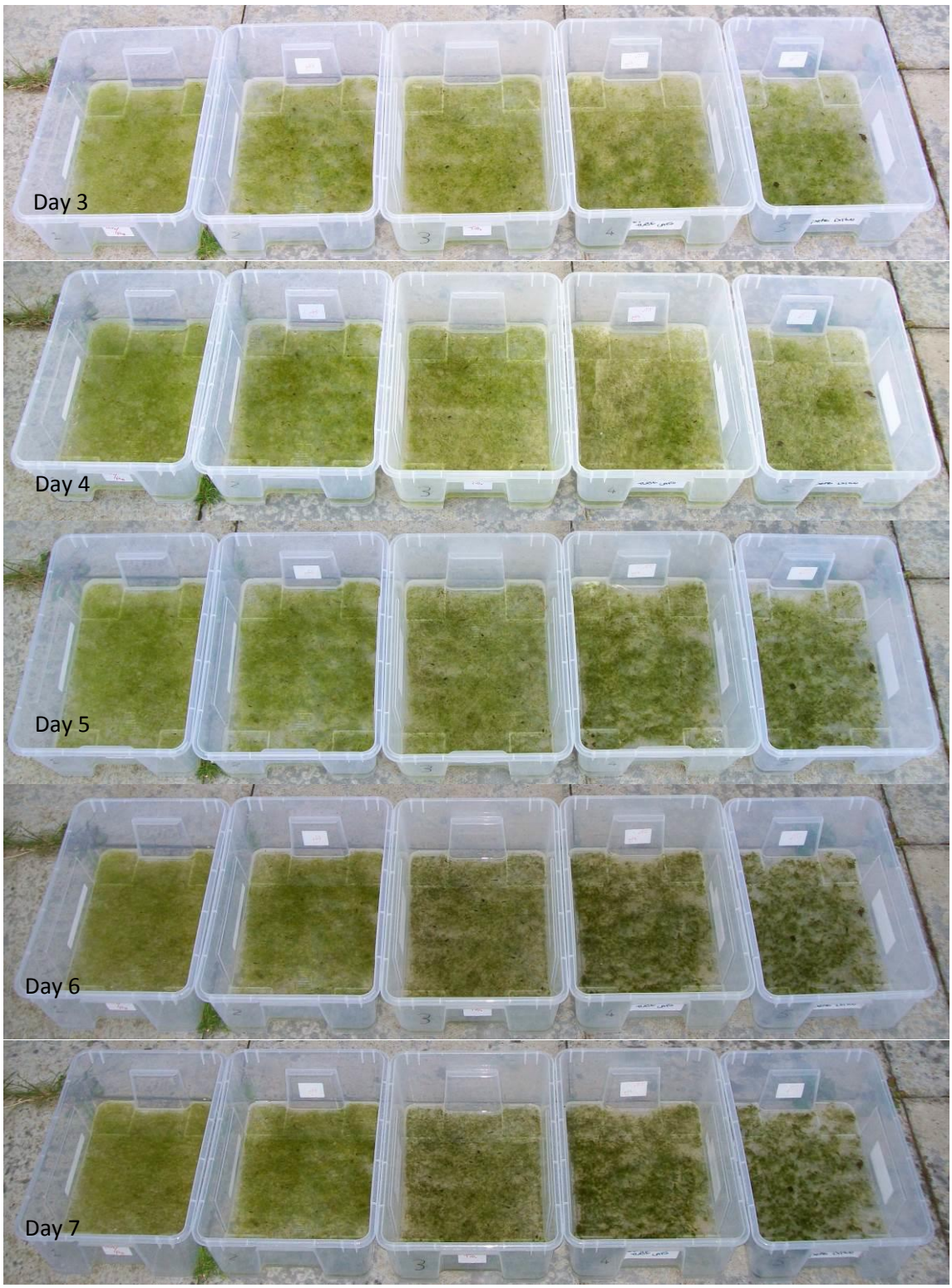
set C as a result of biomass growth yet the measured uptake of NH_4^+ was 98%. Similarly with PO_4^{3-}, from the measured productivity and molecular formula, the calculated PO_4^{3-} uptake was less than that measured with the colorimetric method.

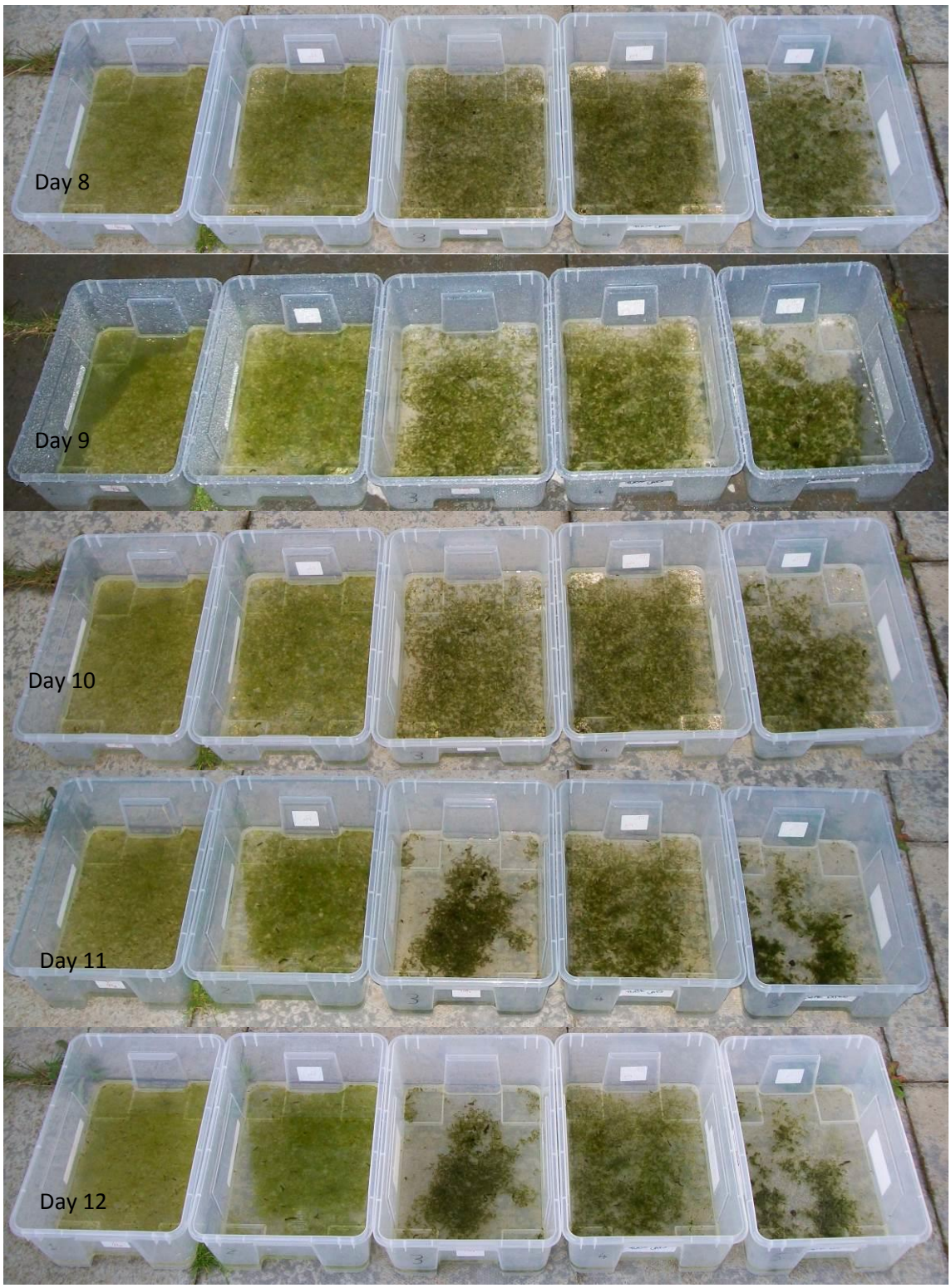
From both sets of results it appears that when the uptake of ammonium and phosphate was measured colorimetrically, more ammonium and phosphate was being recorded as removed than what the algae is capable of removing. The reason may be that some other biological action was taking place and also converting the ammonium and phosphate to other forms. This is likely when the concentrations of ammonium and phosphate in the blank containers are considered (Figures A-1 and A-2 in Appendix A). In each case for the blank containers, the concentrations of both ammonium and phosphate reduced in the containers without biomass but not by the same rate as the container with biomass.

3.3.2 Outdoor cultivation of algal biomass with nutrient addition

The wild freshwater algal biomass that was obtained locally was cultivated in open containers using sterilised pond water and added nutrients with the growth of the biomass observed over time (16 days) and photographs taken regularly (Fig. 3-12).




Day 3
Day 4
Day 5
Day 6
Day 7


Day 8
Day 9
Day 10
Day 11
Day 12

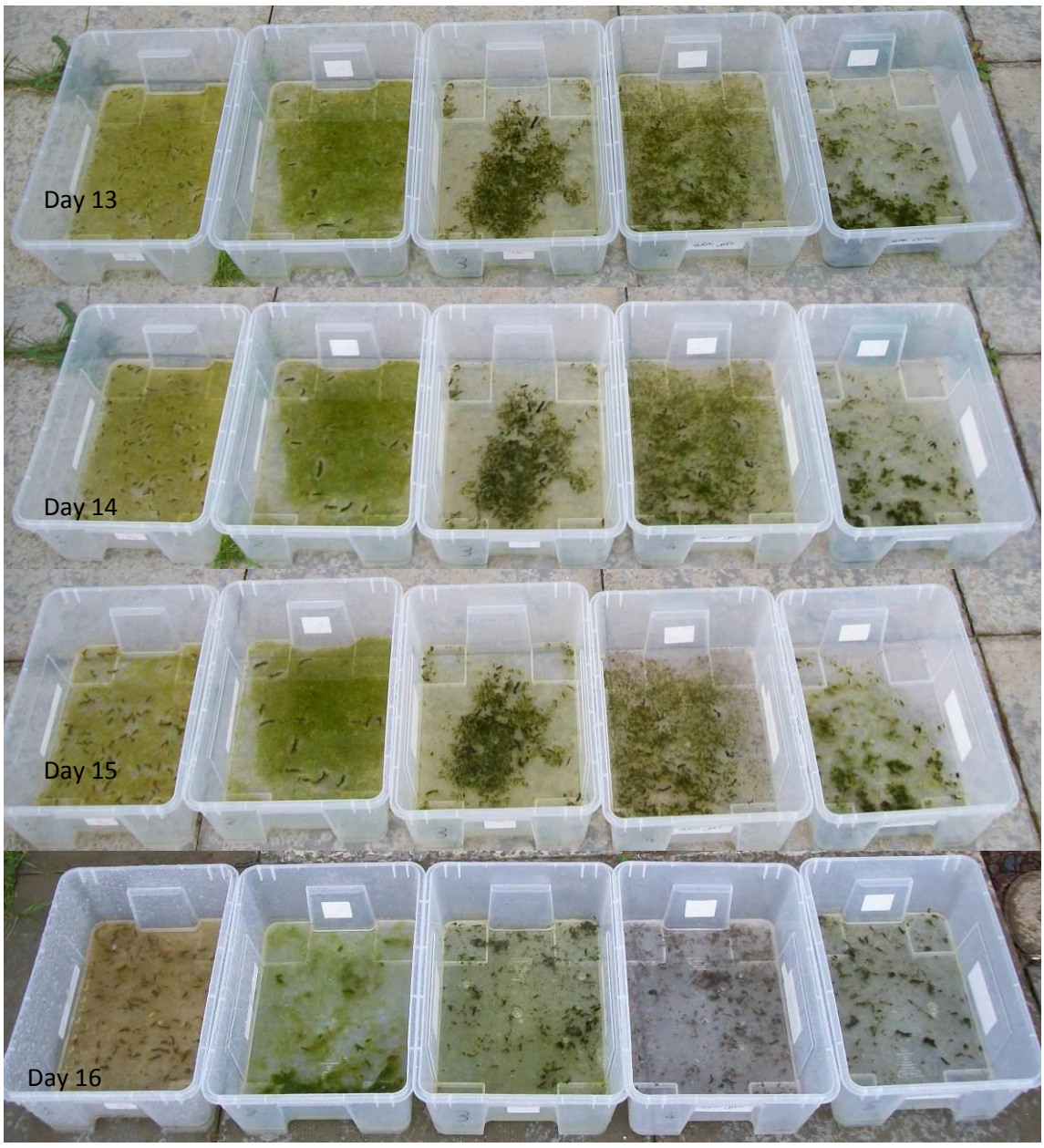


Figure 3-12 Photographic observation of wild algal biomass cultivated in sterilised pond water with added nutrients over a period of 16 days (1: N=0 mg/L, P=0mg/L 2: N=20 mg/L, P=2.9 mg/L 3: N=50 mg/L, P=7.4 mg/L 4: N=80 mg/L, P=11.1 mg/L 5: N=100 mg/L, P= 14.3 mg/L)

From the above figure, it appeared that biomass growth occurred over the first week in each conatinaer after which considerable decline was observed. Some growth in container 2 was evident at the start of the experiment however the biomass also seemed to decline around day 10. The decline was greatest in the containers with the highest nutrient contents and also the container without nutrient addition. Fig. 3-13 displays microscopic images of biomass samples extracted from each container. The biomass cells looked considerably healthier in containers 2 and 3. This suggested that

there is an optimum concentration of nutrient addition (N = 20-50 mg/L and P = 2.9-7.4 mg/L) for good algal growth which has also been confirmed in other literature [141]. It was observed in each case that contamination with parasites had damaged the biomass in each of the containers which is considered one of the main challenges regarding outdoor cultivation of algal biomass [34, 47].

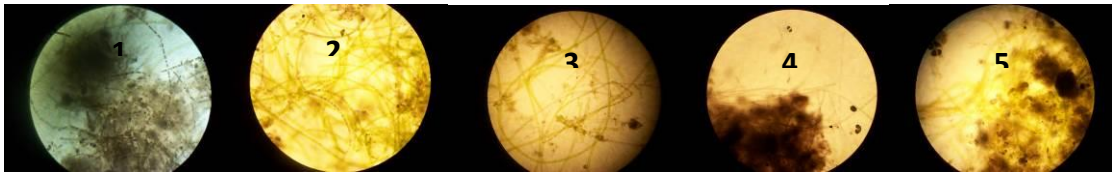


Figure 3-13 Microscopic images of samples of algal biomass extracted from each container (1-5)

3.3.3 Indoor cultivation of pure algal biomass species

A pure strain of *Spirogyra varians* biomass was cultivated in Bold-Basal medium in a controlled laboratory environment, Figure 3-14 displays the flask at day 0 and day 28. Figure A-5 in the appendix shows the flasks at various intervals from day 0 to 28.

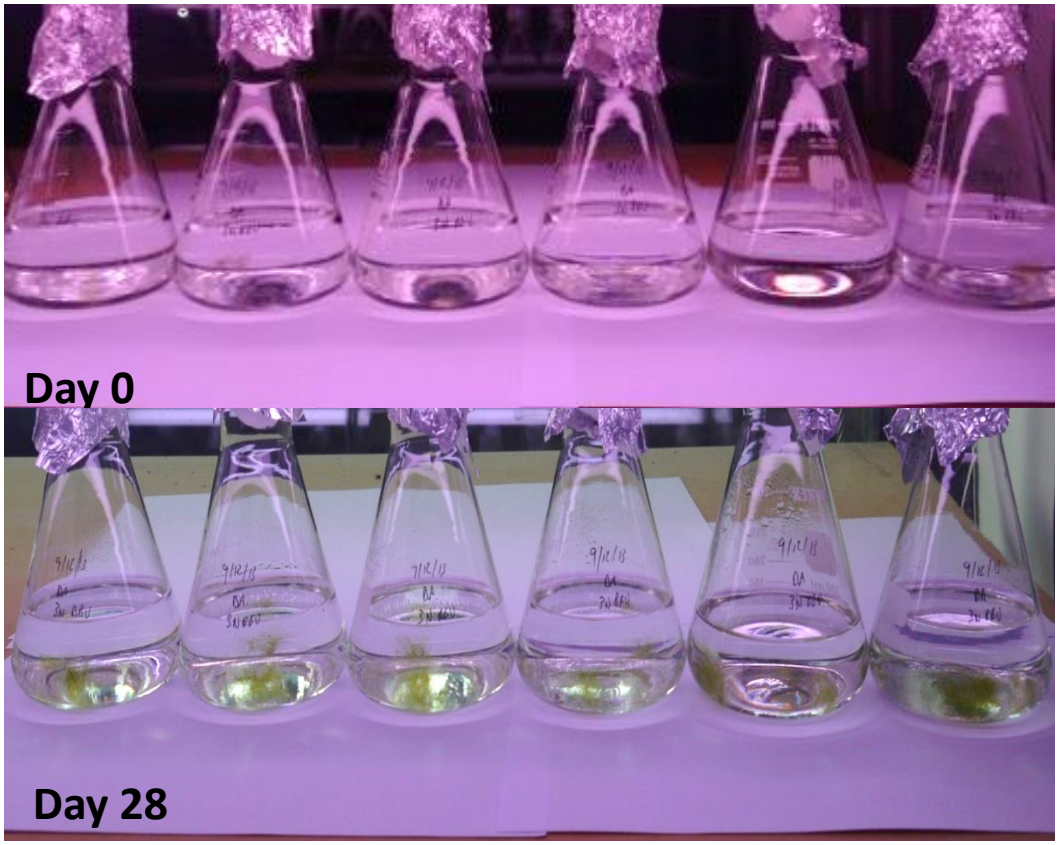


Figure 3-14 Growth of a pure *Spirogyra varians* in Bold-Basal medium

The biomass showed a strong ability to be cultivated in a closed environment under favourable conditions using the Bold-Basal medium without contamination occurring in any of the flasks. Although quantitative measurements were not used it can be observed that growth was successful, suggesting this method of small scale cultivation can be appropriate for developing *Spirogyra* biomass prior to seeding larger scale containers or ponds.

3.3.4 Conversion of biomass to bioethanol

Freshwater algal biomass (*Spirogyra* Sp.) and other biomass types were hydrolysed with enzymes and fermented with yeast to produce bioethanol. The glucose concentrations were measured after 24 and 48 hours of hydrolysis. After 48 hours, yeast (*S. cerevisiae*) was added to the flasks aseptically and ethanol concentrations were then recorded at 2, 4, 12 and 24 hours. Table 3-8 displays the glucose concentrations after 24 and 48 hours of enzymatic hydrolysis treatment.

Table 3-8 Glucose concentrations for each biomass type after 24 and 48 hours of hydrolysis (standard deviation in parentheses)

	Glucose concentration (g/L)		**Glucose/Biomass (%)**	
Biomass	24 hours	48 hours	24 hours	48 hours
Freshwater algae	4.13 (0.23)	4.04 (0.38)	41.26	40.36
Municipal solid waste	3.19 (0.23)	2.74 (0.18)	31.89	27.38
Willow	2.62 (0.17)	2.21 (0.09)	26.21	22.07
Seaweed	1.26 (0.03)	1.23 (0.06)	12.61	12.34
Cellulose	5.86 (0.38)	7.57 (1.02)	58.55	75.67

For each of the biomass types the results show that freshwater algae has the greatest recovery of glucose with the highest recorded value as 4.13 g/L at 24 hours which corresponded to 41.3% of the dry biomass. Municipal solid waste produced the next highest yield (3.19 g/L) followed by willow and seaweed respectively. Compared to alternative studies where hydrolysis of algal biomass has been conducted, the glucose recovery from the biomass is similar and in the range reported in the other studies. Nguyen *et al.* [101] recovered a maximum value of 58% of glucose from *C. reinhardtii,* Ho *et al.* [149] recovered 46.1% from *C. vulgaris* and Rodrigues and Bon [192] recovered 23.3% glucose from *Chlorella homosphaera*.

The results suggest that the freshwater algae used in this study contain a relatively high concentration of easily hydrolysable polysaccharides. In contrast, the polysaccharides in the seaweed appear difficult to hydrolyse which has been noted in similar studies [64, 114]. More advanced techniques for glucose recovery are necessary for the successful hydrolysis of brown seaweed [113]. Willow contains a high concentration of lignin [169], which requires aggressive forms of hydrolysis to

access the polysaccharides. The method of hydrolysis in this study may therefore have been insufficient to fully release the sugars in the biomass [169]. The municipal solid waste contains several different biomass types, where some should be easier to hydrolyse than others: for example the vegetable peelings should be easier to hydrolyse than the woodchips and straw which contain a high percentage of lignin [169]. The glucose recovery from the α-cellulose reached only 75.7%, unlike the other biomass types the glucose concentration had perhaps not peaked and further hydrolysis may have been possible.

In each case (except α-cellulose), the concentration of glucose decreased after 48 hours. The reason for this could be some bacterial activity which is consuming the glucose. As a result of the reduced concentrations the values of ethanol recovery are likely to be slightly less than the highest potential values.

Figure 3-15 below displays the ethanol concentrations obtained from each biomass sample over time. Table 3-9 displays the maximum ethanol concentrations obtained from each biomass type following fermentation and the percentage of ethanol recovered from the final glucose measurement before fermentation and the percentage of ethanol recovered from the dry biomass.

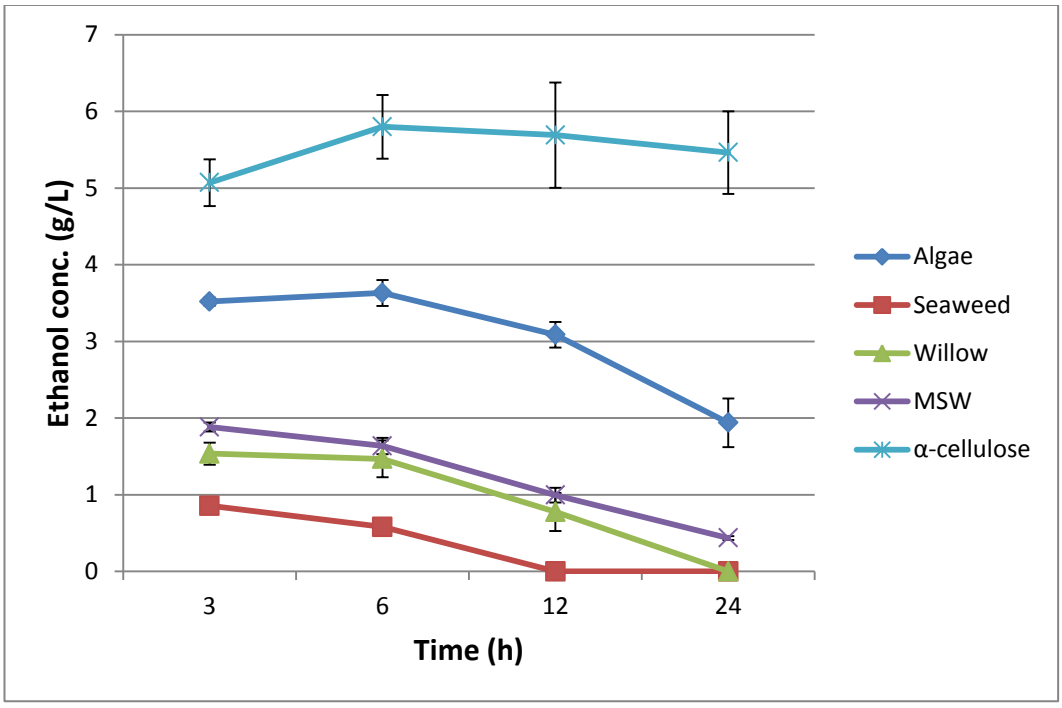


Figure 3-15 Ethanol concentration over time for each biomass type during fermentation

Table 3-9 The maximum ethanol concentrations obtained from each biomass type (standard deviation in parentheses)

Biomass type	Maximum ethanol concentration (g/L)	Ethanol/Glucose (%)	Ethanol/Biomass (%)
Freshwater algae	3.58 (0.17)	88.67	35.78
Municipal solid waste	1.86 (0.06)	68.32	18.56
Willow	1.51 (0.14)	68.64	15.15
Seaweed	0.84 (0.04)	67.78	8.43
Cellulose	5.71 (0.41)	97.58	57.13

For the α-cellulose 98% of the measured glucose was converted to ethanol suggesting the fermentation process was highly efficient. The percentage ethanol recovery from the glucose recorded for the freshwater algae was the highest of the biomass types at 90%. Other studies have recorded similar ethanol production efficiencies from glucose: Ho *et al.* [149] recovered 92.3% of the glucose as ethanol using simultaneous saccharification and fermentation with *C. vulgaris*. In the study by Aikawa *et al.* [173], 86% of measured glycogen was removed during the fermentation process of *Spirulina*. Nguyen *et al.* [101] recovered only 51.2 g ethanol/g glucose for *C. reinhardtii* biomass, however the maximum glucose concentration was high (58% of dry biomass). The ethanol recovered from the glucose for the other biomass types was lower, around 69-70% for each. It may be possible that whith each of these biomass types there were some compounds reducing the conversion efficiency. The final ethanol recovery as a percentage of the dry biomass was therefore greatest for the freshwater algae at 35.8%, almost twice that of the municipal solid waste and over four times that of the seaweed. This ethanol recovery is in the same range as the highest recoveries displayed in figure 1, slightly lower than the recovery recorded by Harun *et al.* [100] for *Chlorococum* sp. (38%) and slightly higher than the recovery recorded by Aikawa *et al.* [173] for *Spirulina* (35%). The ethanol recovery rate also compares well with recovery rates recorded for conventional feedstocks, particularly corn stover and sorghum.

Similarly to the glucose concentrations, the ethanol concentrations reduced over time. The ethanol concentrations reached zero at 12 hours for seaweed and at 24 hours for willow. The ethanol concentration also nearly reached zero for municipal

solid waste after 24 hours. It's possible that bacterial colonies developed in the samples and began to consume the ethanol.

There should be potential to optimise the ethanol production rate from each of the biomass types included in this study, particularly for the seaweed and willow due to the difficulty of hydrolysis of much of the biomass structure. As the polysaccharides in the freshwater algal biomass should be more accessible the benefit of optimisation is likely to be reduced but some increase in ethanol production may be possible. As some of the glucose was lost during the latter part of the hydrolysis process, there will have been a resultant loss in ethanol production. Therefore if glucose loss can be minimised by better control of the hydrolysis the ethanol recovery rate can be higher.

3.3.5 Pyrolysis of algal biomass and municipal solid waste

Table 3-10 displays the product yields for both pyrolysis runs for both the algal biomass and the municipal solid waste. The value in brackets is the percentage yield recovered from the dry mass of biomass. Table 3-11 displays the mean percentage yield and standard deviation of each product for both biomass types.

Table 3-10 Yield of products produced from the pyrolysed biomass for samples I and II (percentages in parentheses)

	Algae I	**Algae II**	**MSW I**	**MSW II**
Dry mass (g)	12.54	12.98	15.75	15.43
Biochar (g)	5.24 (41.8)	5.86 (45.1)	4.76 (30.2)	4.58 (29.7)
Bio-oil (g)	0.12 (1.0)	0.19 (1.5)	0.76 (4.8)	0.95 (6.2)
Light oil (g)	3.51 (30.2)	4.05 (31.2)	4.79 (33)	5.29 (34.2)
Syngas (g)				
H_2	0.043 (0.34)	0.038 (0.29)	0.059 (0.37)	0.030 (0.19)
CO	1.239 (9.88)	1.316 (10.14)	2.378 (15.10)	1.535 (9.95)
CH_4	0.176 (1.41)	0.175 (1.34)	0.414 (2.63)	0.231 (1.50)
C_2H_6	0.106 (0.84)	0.100 (0.77)	0.217 (1.38)	0.114 (0.74)
CO_2	2.602 (20.75)	2.757 (21.24)	3.668 (23.29)	4.965 (32.18)
O_2	0.0010 (0.08)	0.146 (1.28)	0.123 (0.78)	0.021 (0.14)

Table 3-11 Mean percentage product yields for each biomass type (standard deviation in parentheses)

	Algae	**MSW**
Biochar (%)	43.45 (2.33)	29.95 (0.35)
Bio-oil (%)	1.25 (0.35)	5.50 (0.99)
Light oil (%)	30.70 (0.71)	33.60 (0.85)
Syngas (%)		
H_2	0.32 (0.04)	0.28 (0.13)
CO	10.01 (0.18)	12.53 (3.64)
CH_4	1.38 (0.05)	2.07 (0.80)
C_2H_6	0.81 (0.05)	1.06 (0.45)
CO_2	21.00 (0.35)	27.74 (6.29)
O_2	0.68 (0.85)	0.46 (0.45)

The results indicate that the algal biomass produced a greater mass of biochar than the MSW for the conditions tested. The bio-oil, light oil and gas yields are therefore greater for the MSW. The results displayed in tables 3-10 and 3-11, however, contain an inaccuracy. The total mass of the products is greater than the mass of the dry biomass. It is likely that the inaccuracy is a result of the gas measurements. It would appear that the volume of total gas through the system during the experiments is too high probably due to a leak of air entering the volumetric flow meter. Nevertheless using the mass balance of the system, the total mass of gas was calculated by subtracting the masses of oil and biochar from the initial biomass mass. The proportion of gases within the syngas was known by analysis with the mass spectrometer, the percentage yield of masses for each gas were therefore adjusted accordingly. The adjusted results are displayed in table 3-12.

Table 3-12 Mean percentage product yields after syngas mass was reduced by mass balance (standard deviation in parentheses)

	Algae	**MSW**
Biochar (%)	43.45 (2.33)	29.95 (0.35)
Bio-oil (%)	1.25 (0.35)	5.50 (0.99)
Light oil (%)	30.70 (0.71)	33.60 (0.85)
Syngas (%)		
H_2	0.23 (0.06)	0.20 (0.1)
CO	7.23 (1.12)	8.86 (3.13)
CH_4	1.00 (0.20)	1.46 (0.66)
C_2H_6	0.59 (0.14)	0.75 (0.36)
CO_2	15.17 (2.36)	19.30 (3.13)
O_2	0.39 (0.46)	0.33 (0.34)

The results displayed in table 3-12 suggest that freshwater algae is a better candidate for biochar production for the experimental conditions used in the study. The biochar yield from the algal biomass was approximately 13.5% greater than that of the municipal solid waste. In terms of energy recovery, the results show that MSW produced the greater amount of bio-oil, light oil and syngas, although for the energy containing components of the syngas (H_2, CH_4, C_2H_6) the recoveries were similar. Using the energy density values shown in Table 3-13 and the percentage yields recorded (Table 3-12), the bioenergy recovery for one kilogram of freshwater algae was calculated to be 1.35 MJ and 2.38 MJ for MSW.

Table 3-13 Energy content values for each bio-energy product

Product	**Energy content (MJ/kg)**
Bio-oil[1]	17
H_2[2]	120
CH_4[3]	55.5
C_2H_6[3]	47.5

(Note: [1][193]; [2][194]; [3][195])

As a method for energy recovery, the municipal solid waste provided the better feedstock for the conditions tested in this study although the energy recovery was low in comparison to what is considered possible from alternative processing methods such as biodiesel and biogas [30, 36, 90]. Additionally, the usability of the products are unknown and it is likely that further processing would be required [183]. Under the conditions used in the study, the algal biomass produced a greater yield of biochar and therefore appeared to be a better candidate for carbon sequestration. The value of using the algal biochar as a soil amender should also be tested. As this method of pyrolysis required the biomass to be dried prior to processing, the energy required to dry the biomass is an important factor and requires consideration before larger scale tests are conducted.

3.4 General discussion

3.4.1 Cultivation of algal biomass in agricultural effluent

This study was a preliminary study conducted simply to investigate whether cultivation of wild species of algae were able to be cultivated in agricultural effluent. The wild *Spirogyra* species showed excellent potential for cultivation in the agricultural effluent used in the study with good growth observed in each of the dilutions tested. The growth rates were relatively low compared to many studies [30] although no artificial fertiliser was used. Therefore if the concept were to be scaled up the costs and environmental impacts could potentially be lower as a result [27]. The study was conducted inside a laboratory and the biomass therefore did not experience competition from other types of bacteria or parasites which would likely be the case in a scaled up, outdoor scenario. Additionally, a light source was used to supplement the natural light and the laboratory was maintained at room temperature, both of which will have increased biomass productivity. For a larger development the use of artificial light and temperature control would most likely be unviable. Nevertheless in locations where there is a greater intensity of sunlight and a warmer climate than Scotland, it is likely that there would be little difference. The experiment did not include mixing of the water which has been shown to improve biomass productivity by mixing air with water allowing more effective utilisation of CO_2 [196]. Many studies also consider the injection of CO_2 as an important method to increase productivity [197]. Artificial CO_2 was not used in this study. In a scaled up experiment, however, its use could be included and would likely increase productivity.

The locally obtained species, *Spirogyra* sp. showed a good ability to be cultivated in the swine effluent particularly at high concentrations. The results were promising but the experiment should be scaled up and conducted outdoors to check the practicality with regards to sunlight, temperature and competition with other organisms.

3.4.2 Outdoor cultivation of algal biomass with nutrient addition

The outdoor conducted experiment suggested that there was an optimum concentration of nitrogen and phosphorous for the successful cultivation of algae and that too high a concentration could be detrimental. Each of the cultures however

suffered from contamination with other organisms destroying the biomass which highlighted one of the main problems with outdoor cultivation. For larger scale experiments, species of local algae should be selected and cultivated into pure strains before being inoculated in tanks to avoid contamination. However airborne contamination is largely unavoidable.

3.4.3 Indoor cultivation of pure algal biomass species

The indoor cultivation of *Spirogyra varians* proved successful although the growth was not quantitatively measured. This small scale experiment however shows that this specific species which is commonly found in the UK and many parts of the world can be easily cultivated in a closed environment allowing the biomass to be developed and subsequently used to seed larger scale operations.

3.4.4 Conversion of biomass to bioethanol

The conversion of a local species of freshwater algae to bioethanol was conducted to test potential yields. The only study conducted previously examining bioethanol from this particular species yielded poor results [198]. These were improved in the present study by using a mixture of commercial enzymes for the hydrolysis and a high quality strain of yeast for fermentation. The results for the conversion of the locally obtained algal biomass to bioethanol were very promising, particularly in comparison to the other biomass types tested. Little research has been conducted investigating bioethanol recovery from algal biomass but due to the lack of lignin and relatively easily hydrolysed polysaccharides many studies have reported high yields [99-101, 171] and the results in this study were in accordance. As many naturally dominant algal species are filamentous [167] and contain high proportions of carbohydrates, the production of bioethanol is potentially more viable than producing biodiesel or biogas. Indeed, in many cases to maximise biodiesel recovery, specific conditions are required such as low nitrogen growth media. This is not the case for carbohydrates and therefore the cultivation step can be simpler.

Despite high recorded yields the economic and environmental viability of bioethanol production from algal biomass needs to be studied. The hydrolysis and fermentation processes require a high material input (enzymes, yeast, plant etc.) as well as high

energy requirement for grinding the biomass and heating. Additionally, the value of bioethanol is considered to be lower than that of biodiesel due to the lower energy content and reduced demand [199].

3.4.5 Pyrolysis of algal biomass and municipal solid waste

The pyrolysis of algal biomass was considered as an alternative method to recover energy from the biomass and as a method of carbon sequestration. The experiment compared the pyrolysis of locally obtained algal biomass (mainly Spirogyra sp.) and municipal solid waste (produced in laboratory). In terms of the energy products generated (Bio-oil, H_2, CH_4), the municipal solid waste was preferable delivering the higher energy recovery. The algal biomass produced a higher mass of biochar and can therefore be considered a good feedstock for carbon sequestration. However the potential for energy recovery through pyrolysis is low. Additionally, for the experiment the biomass was dried which required a high amount of energy. On a large scale this would greatly reduce the energy balance of the process jeopardising the sustainability.

3.5 Conclusions

This study shows that the swine effluent made a good growth medium for the local wild strain (*Spirogyra* sp.) of algae that was cultivated. For each dilution there was obvious growth but the least diluted effluent gave the best results. The biomass also appeared to remove much of the ammonium and phosphate in the effluent although how much was removed by the algal biomass and how much from other biological actions was not clear. Nevertheless, maximum NH_4^+ and PO_4^{3-} removal rates of 98% and 90% were recorded, respectively. Productivity rates were much lower than can be observed from the cultivation of some microalgae but the results were promising for using swine effluent as a growth medium with the benefit of nutrient removal. Further research should investigate other dilution ratios and cultivation methods as well as identifying the most favourable product recovery from the biomass.

The results are very promising for the recovery of ethanol from the locally obtained freshwater algae (*Spirogyra* sp.). The maximum concentration of ethanol recovered from the algae was similar to the highest recovery rates recorded in other studies for

other species of freshwater algae. The maximum concentration of ethanol was almost twice that of the municipal solid waste which provided the next highest ethanol recovery and was over twice the ethanol concentration recovered from willow and four times the concentration recovered from the seaweed. This local species of freshwater algae therefore makes a highly promising feedstock for ethanol production using conventional processes for conversion. The high ethanol recovery from the glucose concentration for the α-cellulose suggests the method of fermentation was effective and a high conversion of glucose to ethanol was observed for the freshwater algae. The recovery of glucose could potentially be increased by using alternative methods for the biomass hydrolysis such as acid/alkali treatment, microwave treatment or ammonia explosion. Nevertheless, if this species which is naturally dominant in many parts of the world can be cultivated in the open environment without contamination from other species there is a great potential for the conversion of the biomass to bioethanol. It is also possible that the residual biomass could be used as an added value product such as conversion to biogas through anaerobic digestion or used as a fertiliser.

As a method for bioenergy generation from algal biomass, pyrolysis is unlikely to provide a viable technique due to the low production of syngas and bio-oil as well as the heating necessary to dry the biomass. The biomass appears better suited to the production of biochar which has value as a method of carbon sequestration and also as a soil amender. The process could be potentially viable if the cultivation of the biomass has benefits such as treatment of wastewater streams or uptake of CO_2 from flue gases and a high value is placed upon the sequestration of the carbon. The high moisture content of the biomass, however, may reduce the practicality of this method. Further work is required to test alternative conditions for pyrolysis and to check the economic and environmental viability.

The main conclusions from this chapter are that:

- A common local species of algae (*Spirogyra* sp.) was able to be cultivated in various dilutions of swine effluent;
- Outdoor cultivation presents challenges related to competition from other organisms;

- High bioethanol recoveries (up to 38%) are possible from a locally obtained species of algae (*Spirogyra* sp.);
- Pyrolysis of the locally obtained species yielded low energy recovery but may be suitable as a method for carbon sequestration.

The results from this chapter suggest that cultivation of localised species of algae in wastewater should be considered as a method of nutrient removal and energy recovery. Additionally, the high yields of bioethanol recovered from the biomass offer an alternative bioenergy recovery technique to more widely researched biodiesel production. The use of algal cultivation for nutrient recovery and bioenergy generation should therefore be considered and tested on a larger scale with the sustainability being assessed using life cycle assessment methodology. The following chapter investigates the use of algal cultivation as a method of nutrient recovery in a wastewater treatment plant. The sustainability is determined and compared to a conventional method of nutrient removal.

4 A life cycle assessment comparison of freshwater algal cultivation and conventional methods for nutrient removal of wastewater

4.1 Introduction

The wastewater treatment industry is facing two important requirements: to improve treatment efficiencies [200] and to minimise environmental impacts [1]. This chapter investigates the sustainability of two treatment methods to lower nutrient loading in the effluent of a wastewater treatment plant in Israel using a life-cycle assessment (LCA) approach. One method used conventional treatment techniques for enhanced nutrient removal and the other used a novel method to reduce nutrient loading whilst simultaneously producing biomass for energy generation.

In an arid country like Israel, water is an extremely precious resource and wastewater is recycled where possible for irrigation whilst the remainder is discharged [201]. As a result, wastewater treatment plants do not provide full nutrient removal which has led to eutrophication of streams and rivers in areas where the effluent is used for irrigation or discharged [202]. To tackle this issue, the Israeli Ministry of Environmental Protection upgraded the effluent quality standards for treatment plants [203]. The standards depend upon whether the wastewater is used for irrigation or simply discharged with discharge limits being stricter. The discharge limit of total nitrogen is 25 mg/L for irrigation and 10 mg/L for discharge to rivers [203]. Wastewater treatment plants must therefore upgrade their treatment methods to comply with these regulations.

There are various methods to reduce the nitrogen and phosphorous content of wastewaters. In wastewater treatment plants, biological nutrient removal is a common method to reduce the concentration of nitrogen and phosphorous in the wastewater [200]. Biological removal of nutrients requires relatively complex biochemical reactions to occur using different microbial communities [200]. To allow these different microbes to develop, different conditions are required. For the removal of nitrogen, the most common process is nitrification-denitrification [200]. Nitrogen in wastewater is mainly in the form of ammonia. For removal to occur the

nitrogen must be converted to nitrogen gas, $N_{2,}$, firstly by oxidation to nitrite and nitrate and then conversion to nitrogen gas by denitrification [200]. The removal of phosphorous requires the development of polyphosphate accumulating organisms (PAOs) which uptake the phosphorous and can be removed through sludge wasting [200]. Anaerobic and aerobic conditions are necessary for the release of phosphorous and the subsequent uptake by PAOs [200].

Conventional nutrient removal can be expensive and energy intensive [200]. Cultivation of algal biomass offers an alternative to conventional nutrient removal [27]. To develop, algal biomass must have access to both nitrogen and phosphorous. During their cultivation the biomass assimilates the nitrogen and phosphorous and the concentration in the wastewater therefore reduces. The production of algal biomass is also beneficial as it can be used for recovery of energy through various different pathways. The high productivity and energy yields that have been proven for some species of algal biomass has prompted much research in the area where the cultivation of algal biomass solely for the recovery of energy is possible. However the sustainability is very much in doubt due to the high intensity of many of the involved processes regarding energy and material use [10, 27, 35]. One of the main sources of energy demand and environmental impacts is the consumption of fertiliser [27]. The use of wastewater, however, as a source of nutrients avoids the consumption of fertiliser and provides an added benefit as a method of tertiary wastewater treatment [10]. There have been studies published recently considering the use of wastewater as a source of nutrients for the cultivation of algal biomass [34, 133, 204] but in most cases the focus has not been on whether the system is an improvement to alternative treatment methods. Sturm *et al.* [204] recorded a net positive energy balance for the recovery of biodiesel from algal biomass cultivated in wastewater. Their study used data produced at their own facility but did not consider the cumulative energy demand of required materials nor other products from the biomass other than biodiesel.

This study used a life cycle assessment (LCA) approach to model the energy analysis and environmental impacts of using two possible enhanced nutrient removal scenarios for Haifa Waste Water Treatment Plant (WWTP):

S1 Nutrient removal using the A_2O (Anaerobic/Anoxic/Aerobic) process

S2 Nutrient removal using algal biomass cultivation

For both scenarios, the model investigated the energy required to reduce the total nitrogen concentration to the maximum level of 10 mg/L and the total phosphorous concentration to 1 mg/L (discharge standards in Israel) as well as considering several environmental impacts. In both scenarios energy was produced: In scenario 1, energy was produced as a result of biogas generation from anaerobic digestion of sludge, whilst in scenario 2, energy was generated as a result of sludge digestion and additionally through processing of the algal biomass.

The energy analysis considered the energy balance of treating the wastewater by subtracting the energy consumed from the energy produced. The energy consumed was calculated as the cumulative energy demand of each of the processes including the direct energy consumption and the materials. For the environmental impacts, the impacts upon three common environmental impact categories were determined: global warming potential, acidification potential and eutrophication potential. The global warming potential provides an indication of the potential impact to climate change based on the equivalent CO_2 emissions and the acidification and eutrophication potentials indicate the environmental risks to the local environment (atmospheric, soil and water) based on equivalent emissions of SO_2 and PO_4 respectively.

The study examined the impacts for each scenario for one day of wastewater treatment using data from relevant studies, reports and calculations. The wastewater characteristics were based on data published in a recent report by Haifa WWTP [205]. The impact values were determined using data from Ecoinvent [106] and computed using OpenLCA software [206]. Based on the results, the study aims to provide an indication of the most environmentally preferred scenario for upgrading the current treatment of Haifa WWTP to allow for improved nutrient reduction.

4.2 Methodology

This study used life-cycle assessment (LCA) modelling to determine the energy balance and environmental impacts of both scenarios in accordance with ISO 14040. The goal and scope of the study were determined followed by completion of the inventory analysis performed by splitting both scenarios into their respective stream of unit processes and calculating the mass balance and the material and energy inputs. The impact assessment was then conducted by determining the impacts of each of the processes by computing the inputs and calculating the contribution to each impact.

4.2.1 Functional unit

The functional unit used was one day of WWTP operation, the input values therefore corresponded to the volume of wastewater treated in one day. The daily volume of wastewater treatment treated was taken to be 120,000 m^3 based on the future capacity of Haifa WWTP following upgrading. The material and energy inputs for both scenarios were therefore calculated based on a throughput of 120,000 m^3.

4.2.2 Goal and scope

The goal of the study was to identify which scenario would provide the most sustainable form of nutrient removal for Haifa WWTP considering the energy balance and the environmental impacts. The purpose was also to investigate which processes and inputs contribute the most to the energy demand/impact category. Although the data is used for Haifa WWTP, the results are potentially applicable to other wastewater treatment plants. The study considered two scenarios, the first being the current treatment set-up with the inclusion of a more advanced nutrient removal process, the A_2O process, in place of the mechanical aeration. The second was the existing treatment process stream with the effluent being sent to algal ponds for further nutrient removal after which the algal biomass was assumed to be harvested and converted to an energy carrier. This scenario included several, alternative bioenergy pathways, they were: A Biodiesel and biogas production, B Bioethanol and biogas production, C Biogas production and D Biodiesel, bioethanol and biogas production. In both scenarios it was assumed that the biogas produced

from the anaerobic digestion of sewage sludge was used in a co-generation biogas turbine to produce electricity and heat which were returned to the system to cover a proportion of the energy demand. Additionally, for scenario 2, the biogas produced from the algal biomass was assumed to be used in the same way. Where biodiesel and bioethanol were produced, they were assumed to replace conventional methods for their production. Where an electricity deficit was calculated, the national electricity grid was assumed to be used which was modelled using available data from the Ministry of Infrastructures [207].

The energy balance was determined by calculating the total energy produced from the system (the lower heating value of the energy products and cumulative energy demand of co-products) and subtracting the cumulative energy demand (CED) of each of the processes within the system. The cumulative energy demand was calculated using data from Ecoinvent [106] and computed using OpenLCA [206] and considered only the non-renewable fossil energy. The environmental impacts were calculated using the CML 2001 [208] method, the impact categories that were considered were the global warming potential over 100 years (kg CO_2-Eq), the acidification potential-generic (kg SO_2-Eq) and the eutrophication potential-generic (kg PO_4-Eq).

4.2.3 Models

The two scenarios were split into their process streams to be studied, Figure 1 displays the unit processes included in each scenario and the different bioenergy pathways for scenario 2. Both scenarios share a common primary treatment of clarification however the secondary treatment and subsequent processes differ. All processing was assumed to take place on the site of the wastewater treatment plant.

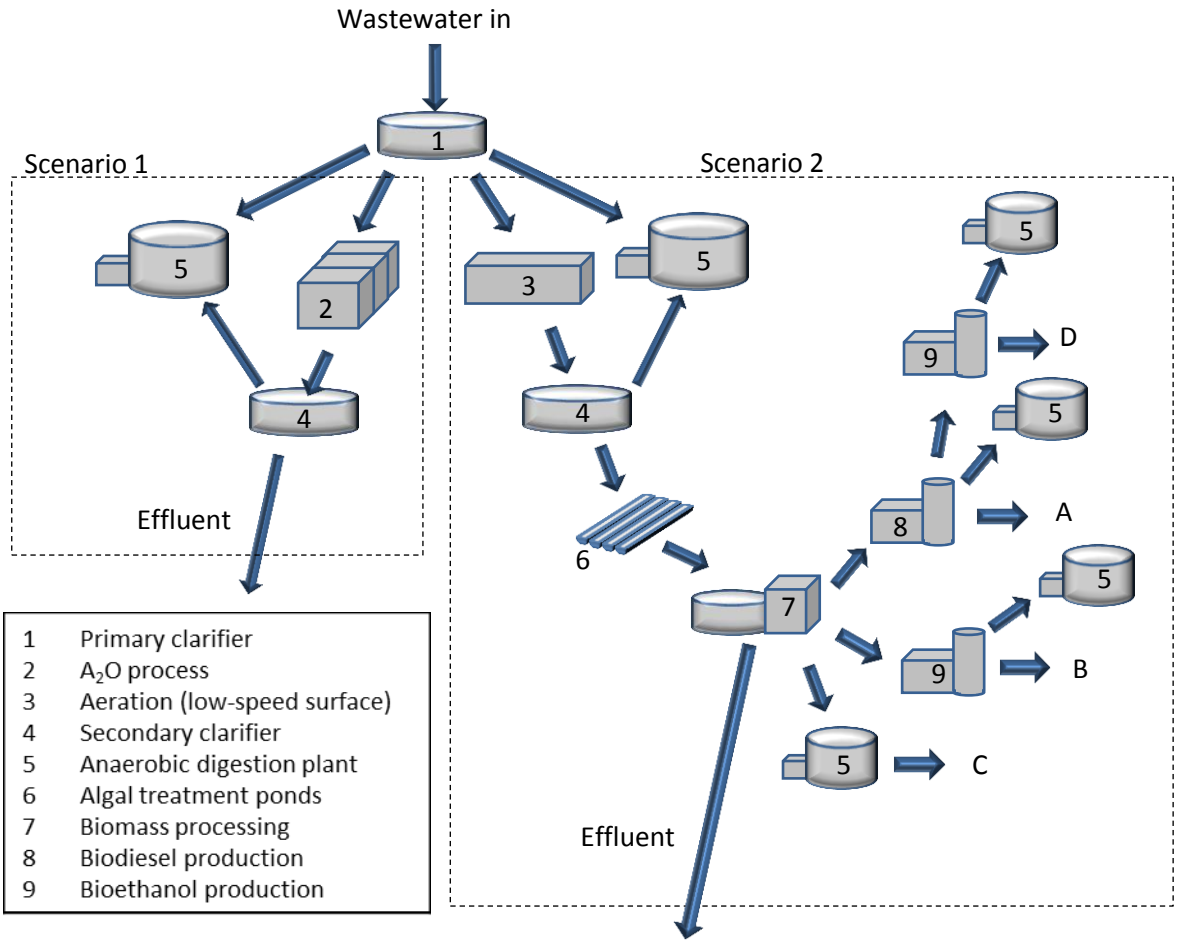


Figure 4-1 Process streams for scenario 1 and scenario 2

4.2.3.1 Scenario 1

Scenario 1 considered the existing set-up of the wastewater treatment plant with the use of the A_2O process for nutrient removal as a replacement to the current activated sludge process. In the process that was common to both scenarios the wastewater enters the primary clarifier where sludge is settled from the water and sent to the anaerobic digestion facility. The effluent is then pumped to the A_2O process for nutrient removal after which the effluent is sent to the secondary clarifier for sludge removal. The sludge is pumped to the anaerobic digester and the effluent is discharged. Biogas produced from the primary and secondary sludge was assumed to be converted to electricity and heat in a biogas turbine. The produced energy was assumed to be returned to the system to be used where necessary. The electricity

deficit was assumed to be made up by using the national electricity grid and the surplus heat from the co-generation turbine was assumed not to be used.

4.2.3.2 Scenario 2

Scenario 2 considered the existing set-up of the wastewater treatment plant with no changes to the process stream. Following the secondary clarification however the effluent was assumed to be sent to algal ponds for further nutrient removal. Similarly to scenario 1 the first step is primary clarification where the sludge is pumped to the anaerobic digester. The effluent is then sent to the aeration tank after which the secondary clarifier removes the sludge which is sent to the anaerobic digester and the effluent to the algal treatment ponds. The effluent is retained in the algal ponds until the nutrient concentrations are sufficiently low. The broth is then sent to a flocculation tank to remove the algal biomass. The effluent is discharged and the biomass is sent to a centrifuge for dewatering. The concentrated biomass was then processed to bioenergy where four different pathways were examined. These were the conversion of algal biomass to:

A Biodiesel and biogas
B Bioethanol and biogas
C Biogas only
D Biodiesel, bioethanol and biogas

For the production of biodiesel, the process was modelled on that used for the production of biodiesel from soy bean from Ecoinvent [106]. The production of bioethanol was modelled using data based on the conversion of corn to bioethanol from Ecoinvent [106] and the anaerobic digestion of the biomass to biogas was modelled using relationships determined for the digestion of sludge in wastewater treatment [200]. In each case, a proportion of the electricity and heat generated from the biogas produced was assumed to be returned to the system to cover the energy needs. Where there was a deficit of electricity the national grid was assumed to cover the shortfall and where there was a surplus the electricity was assumed to be exported. Any surplus of heat was assumed not to be used. The residual biomass (digestate) from each process stream was assumed to be used as fertiliser based on the mass of residual biomass, the nitrogen and phosphorous content of the biomass

and the mineralisation rate of the nitrogen and phosphorous (Appendix B.4). The digestate was first assumed to be separated into solids and liquids using centrifugation. The fertiliser was assumed to offset the production of ammonium sulphate and single superphosphate, the impacts of which were calculated using data from Ecoinvent [106].

4.2.4 Data acquisition

The wastewater characteristics were taken from data reported by the wastewater treatment plant operators [205] (Appendix B.4). The data related to the energy consumption of the wastewater treatment processes was calculated using standard wastewater treatment processing relationships as well as relationships determined in literature and reports. For the cultivation and processing of algal biomass in scenario 2, the inventory data was obtained from published literature. The data used for modelling the Israel national electricity grid was obtained from the Ministry of National Infrastructures [207] (Appendix B.2). The cumulative energy demand and environmental impacts were calculated using data from the Ecoinvent database and processed using the CML 2001 method with OpenLCA and the results compiled on Microsoft excel spreadsheets.

4.3 Life cycle inventory

The information and data related to the life cycle inventory for each scenario are detailed in the supplementary material. Table 4-1 displays the inputs to scenario 1 as well as the energy generated, consumed and the energy surplus/deficits. Table 4-2 displays the inputs to scenario 2 prior to the separate process streams. Tables 4-3,4-4,4-5 and 4-6 display the inputs and energy generated, consumed and the energy surplus/deficit for process streams S2 A, S2 B, S2 C and S2 D, respectively.

Table 4-1 Values of energy/material inputs and the values of energy generated, consumed and the surplus/deficit for Scenario 1

Energy and material inputs		
Primary clarifier	Scraper	502 kWh
A_2O process	Anaerobic tank mixer Anoxic tank mixer Aerobic tank diffuser Concrete, block Concrete, normal Pumping	680 kWh 680 kWh 39,919 kWh 446 kg 0.316 m^3 11,741 kWh
Secondary clarifier	Scraper	497 kWh
Sludge thickener	GBT	63 kWh
Anaerobic digester	Mixing Heating	5,162 kWh 83,647 MJ
De-watering	Centrifuge	1,343 kWh
Biogas co-generation	Lubricating oil	15.4 kg
Energy generated, consumed and surplus		
Energy generated	Biogas generated Electricity generated Heat generated	23,148 m^3 45,514 kWh 281,619 MJ
Energy consumed	Electricity consumed Heat consumed	53,503 kWh 92,012 MJ
Energy surplus/deficit	Electricity Heat	-7,989 kWh 189,607 MJ

Table 4-2 Energy and material inputs for scenario 2 prior to the individual process streams

Primary clarifier	Scraper	507 kWh
Activated sludge	Mixers	79,483 kWh
Secondary clarifier	Scraper	502 kWh
Sludge thickener	GBT	75 kWh
Anaerobic digester	Mixing Heating	5,908 kWh 105,462 MJ
De-watering	Centrifuge	1,537 kWh
Biogas co-generation	Lubricating oil	18 kg
Algal cultivation Pond	Pumping Paddlewheel Superphosphate Flue gas injection Concrete, block Concrete, normal	4,784 kWh 27,404 kWh 1,812 kg 1,632 kWh, 1,620 kWh, 1,760 kWh, 1,519 kWh[1] 8,894 kg 42,3 m^3
Flocculation	Pumping Mixing Flocculant Concrete, block Concrete, normal	2,989 kWh 190 kWh 5,555 kg 40.2 kg 0.017 m^3
Sedimentation	Pumping Scraper Concrete, block Concrete, normal	688 kWh 279 kWh 46.1 kg 0.021 m^3
Centrifugation	Pumping Centrifuge Steel, chrome	22 kWh 4,740 kWh 8.7 kg
Homogenization	Wet grinding attritor Steel, chrome	1,775 kWh 0.25 kg

(Note: [1]Values for S2 A, B, C and D respectively)

Table 4-3 Values of energy/material inputs and the values of energy generated, consumed and the surplus/deficit for process stream S2 A

Energy and material inputs		
Lipid extraction	Electricity	2,326 kWh
	Heat	40,483 MJ
	Hexane	8.9 kg
	Phosphoric acid	7 kg
	Tap water	6,499 kg
	Oil mill[1]	1.3×10^{-5}
Trans-esterification	Electricity	983 kWh
	Heat	10,476 MJ
	Hydrochloric acid	52 kg
	Methanol	1,288 kg
	Phosphoric acid	129 kg
	Tap water	310 kg
	Trans-esterification plant	1.06×10^{-6}
Anaerobic digestion	Mixing	2,114 kWh
	Heat	34,305 MJ
	Concrete, block	119 kg
	Concrete, normal	0.028 m^3
Biogas co-generation	Components	1.51×10^{-3}
	Lubricating oil	8.8 kg
Energy generated, consumed and surplus/deficit		
Energy generated	Biodiesel generated	12,321 kg (458,323 MJ)
	Biogas generated	39,817 m^3
	Electricity generated	78,277 kWh
	Heat generated	484,336 MJ
Energy consumed	Electricity consumed	86,474kWh
	Heat consumed	227,899 MJ
Energy surplus/deficit	Electricity	-8,197 kWh
	Heat	256,437 MJ

(Note: [1]Fraction of an oil mill with a capacity of 20,000 tonnes of oil per year)

Table 4-4 Values of energy/material inputs and the values of energy generated, consumed and the surplus/deficit for process stream S2 B

Energy and material inputs		
Fermentation/ Distillation	Electricity	12,143 kWh
	Heat	264,375 MJ
	Ammonium sulphate	236 kg
	Diammonium phosphate	236 kg
	Soda powder	880 kg
	Sulphuric acid	481 kg
	Bioethanol plant[1]	1.21×10^{-5}
Bioethanol upgrading	Electricity	61 kWh
	Heat	6,989 MJ
	Bioethanol plant	5.3×10^{-11}
Anaerobic digestion	Mixing	2,068 kWh
	Heat	36,918 MJ
	Concrete, block	116 kg
	Concrete, normal	0.028 m^3
Biogas co-generation	Components	1.48×10^{-3}
	Lubricating oil	8.6 kg
Energy generated, consumed and surplus/deficit		
Energy generated	Bioethanol generated	6,957 kg (195,500 MJ)
	Biogas generated	39,528 m^3
	Electricity generated	77,710 kWh
	Heat generated	480,828 MJ
Energy consumed	Electricity consumed	93,406 kWh
	Heat consumed	413,7444 MJ
Energy surplus/deficit	Electricity	-15,697 kWh (-56,509 MJ)
	Heat	67,083 MJ

(Note: [1]Fraction of a bioethanol plant with a capacity of 90,000 tonnes of ethanol per year)

Table 4-5 Values of energy/material inputs and the values of energy generated, consumed and the surplus/deficit for process stream S2 C

Energy and material inputs		
Anaerobic digestion	Mixing Heat Concrete, blocks Concrete, normal	2,610 kWh 42,352 MJ 147 kg 0.029 m^3
Biogas co-generation	Components Lubricating oil	1.87×10^{-3} 10.9 kg
Energy generated, consumed and surplus/deficit		
Energy generated	Biogas generated Electricity generated Heat generated	42,947 m^3 84,417 kWh 522,332 MJ
Energy consumed	Electricity consumed Heat consumed	82,571 kWh 152,049 MJ
Energy surplus/deficit	Electricity Heat	1,847 kWh (6,648 MJ) 370,283 MJ

Table 4-6 Values of energy/material inputs and the values of energy generated, consumed and the surplus/deficit for process stream S2 D

Energy and material inputs		
Lipid extraction	Electricity	2,326 kWh
	Heat	40,483 MJ
	Hexane	8.9 kg
	Phosphoric acid	7 kg
	Tap water	6,499 kg
	Oil mill[1]	1.3×10^{-5}
Trans-esterification	Electricity	983 kWh
	Heat	10,476 MJ
	Hydrochloric acid	52 kg
	Methanol	1,288 kg
	Phosphoric acid	129 kg
	Tap water	310 kg
	Trans-esterification plant[2]	1.06×10^{-6}
Fermentation/ Distillation	Electricity	9,836 kWh
	Heat	214,144 MJ
	Ammonium sulphate	191 kg
	Diammonium phosphate	191 kg
	Soda powder	713 kg
	Sulphuric acid	477 kg
	Bioethanol plant[3]	9.8×10^{-6}
Bioethanol upgrading	Electricity	61 kWh
	Heat	6,989 MJ
	Bioethanol plant[3]	5.3×10^{-11}
Anaerobic digestion	Mixing	1,675 kWh
	Heat	29,904 MJ
	Concrete, block	94 kg
	Concrete, normal	0.027 m^3
Biogas co-generation	Components	1.20×10^{-3}
	Lubricating oil	7.0 kg
Energy generated, consumed and surplus/deficit		
Energy generated	Biodiesel generated	12,321 kg (458,323 MJ)
	Bioethanol generated	6,957 kg (195,500 MJ)
	Biogas generated	37,048 m^3
	Electricity generated	72,843 kWh
	Heat generated	450,717 MJ
Energy consumed	Electricity consumed	95,709 kWh
	Heat consumed	441,200 MJ
Energy surplus/deficit	Electricity	-22,865 kWh (-82,316 MJ)
	Heat	189,607 MJ

(Note: [1]Fraction of an oil mill with a capacity of 20,000 tonnes of oil per year, [2]Fraction of a trans-esterification plant with a capacity of 22,000 tonnes of methyl ester per year, [3]Fraction of a bioethanol plant with a capacity of 90,000 tonnes of bioethanol per year)

4.4 Results and discussion

The purpose of this study was to investigate the most sustainable method of nutrient removal comparing conventional nutrient removal and a more novel method using algal cultivation ponds. The methods used to assess the sustainability were energy analysis considering the cumulative energy demand and produced energy as well as the global warming, acidification and eutrophication potentials.

4.4.1 Energy balance

The energy balance was calculated by summing the cumulative energy demand of each of the processes for each scenario and process stream and subtracting these from the energy (lower heating value) produced as biodiesel, bioethanol, exported electricity and fertiliser (offset CED). Figure 4-2 (a) displays the energy balance of each of the scenarios and each process stream for scenario 2. Figure 4-2 (b) displays the contribution analysis for the cumulative energy demand and the energy produced for each scenario and process stream. Figure 4-3 displays a more detailed representation of the process contributions to the cumulative energy demand only for each scenario.

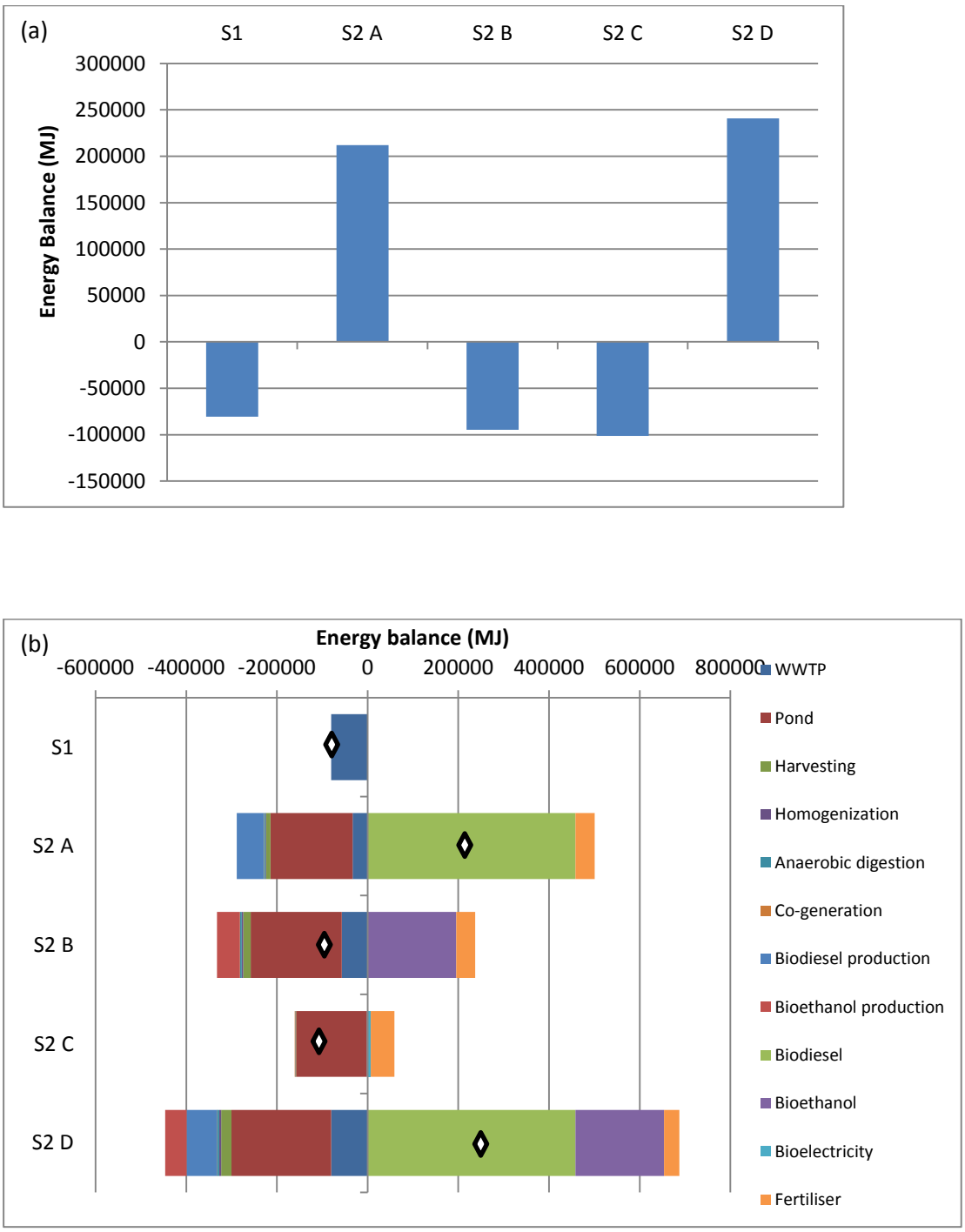


Figure 4-2 (a) Energy balance and (b) contribution analysis for each scenario and process stream

(Note: the diamonds represent the net energy balance)

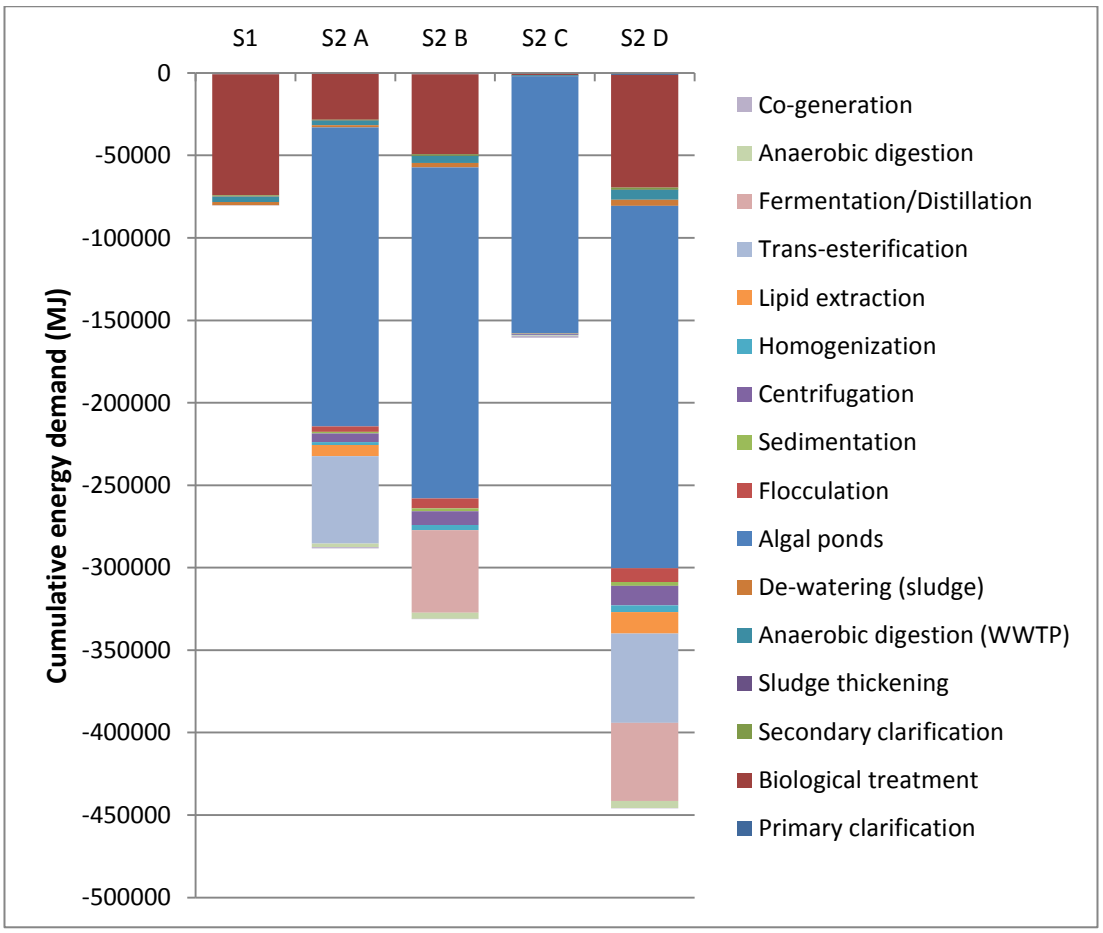


Figure 4-3 The contribution of the unit processes to the cumulative energy demand for all scenarios and process streams

Figures 4-2 (a) and 2 (b) show that scenario 1 had a net negative energy balance (-80,487 MJ) because insufficient biogas was produced to cover the electricity requirements and therefore no energy was exported. As figure 4-3 shows, the greatest contribution to the cumulative energy demand was the biological treatment which accounted for 91.3% of the CED. For the biological treatment, the diffuser required the greatest energy demand at 66.2% of the total CED followed by the wastewater pumping (13.9%). The anaerobic digestion of the sludge accounted for 3.9% of the total CED, 97% of which was for the mixing. Scenario 2 A produced a positive energy balance of 212,148 MJ as a result of the energy contained within the produced biodiesel (458,323 MJ) and a small contribution from the produced fertiliser (42,657 MJ). Figure 3 shows that the energy demand of the biological treatment was low in comparison to S1 (10.2%), the greatest energy demand was from the algal ponds (70.8%). The use of fertiliser (superphosphate) to supplement

the phosphorous limitation was the greatest energy input accounting for 30.9% followed by the use of concrete (22.6%). The transesterification of the biomass accounted for 18.3% of the cumulative energy demand. Due to biogas produced from the wastewater sludge and algal biomass being used for the electricity and heat requirements, for the transesterification process the use of methanol accounted for 91.1% of the energy demand. Both bioethanol production (S2 B) and biogas production only (S2 C) produced negative energy balances of -94,779 MJ and -107,863 MJ respectively. In comparison to biodiesel, the recovery of bioethanol was low and less biogas was produced from the residual biomass meaning more electricity was required to be used from the national grid leading to a higher cumulative energy demand for each process using electricity. Similarly to S2 A, the algal cultivation ponds had the greatest energy demand (60.5%) and again superphosphate had the greatest demand (26.9%) although due to the lower biogas yield, the energy demand of the electricity consumption for the paddlewheel operation was greater (13.9%). For S2 C, due to the high electricity requirements, none of the electricity generated from the biogas co-generation was exported which led to a negative energy balance. The cumulative energy demand of the whole system was low because the biogas co-generation almost covered the total energy requirements. In this case the algal cultivation ponds accounted for 98.3% of the total CED due to the superphosphate consumption (55.6%) and the concrete use (40.6%). The energy balance for S2 D was positive and slightly higher than S2 A at 240,958 MJ. The production of both biodiesel and bioethanol increased the value of energy produced by a greater margin than the increase in cumulative energy demand for the extra processing. The proportions of the energy demand for each process was similar to the S2 A and S2 B with the algal cultivation being the most energy demanding process (60.0%). The fermentation and distillation process has a lower energy demand (12.9%) compared to lipid extraction and transesterification (18.3%) because the main energy required is heat as opposed to electricity.

4.4.2 Environmental impacts

The environmental impacts considered were global warming potential, acidification and eutrophication. For global warming potential, the carbon contained in the fraction of biomass that was converted to fertiliser was negated from the total CO_2-

Eq emissions. Additionally, the CO_2-Eq emissions avoided through the production of biodiesel, bioethanol and fertiliser were negated. This was also conducted for acidification and eutrophication for SO_2-Eq and PO_4-Eq emissions respectively. The total values for each environmental impact category are displayed in table 4-7. The contribution of each process to the environmental impacts are displayed as Figures 4-4 (a), (b) and (c) for global warming potential, acidification and eutrophication respectively.

Table 4-7 Environmental impact values for each scenario and process stream

Process stream	**Global warming potential (kg CO_2-Eq)**	**Acidification (kg SO_2-Eq)**	**Eutrophication (kg PO_4-Eq)**
S1	11,839	60.6	15.0
S2 A	-56,872	85.9	-95.0
S2 B	-45,323	125.0	-42.9
S2 C	-75,924	107.5	22.6
S2 D	-34,088	84.8	-165.9

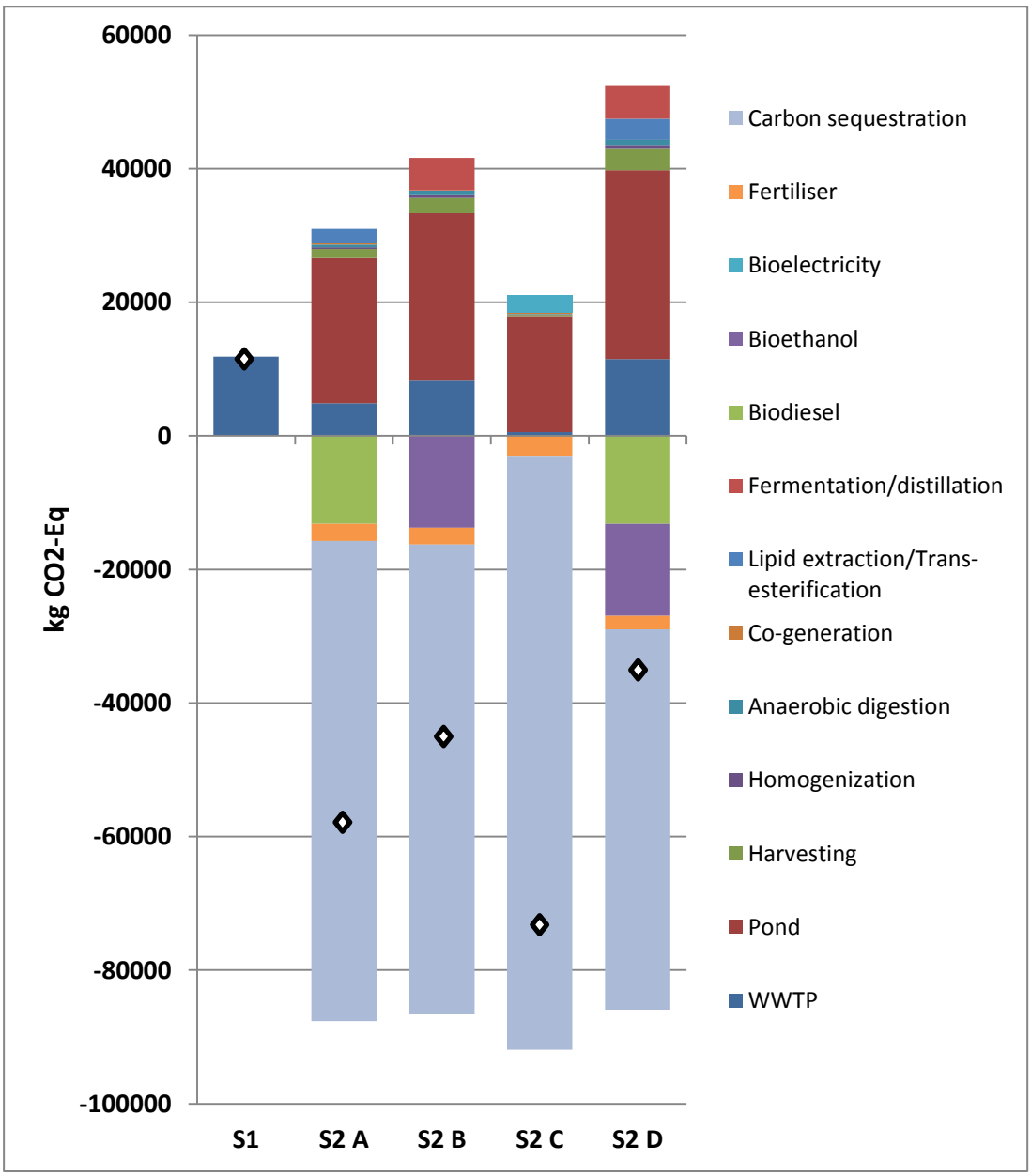


Figure 4-4 The contribution of the unit processes to the global warming potential for each scenario and process stream

(Note: the diamonds represent total net value)

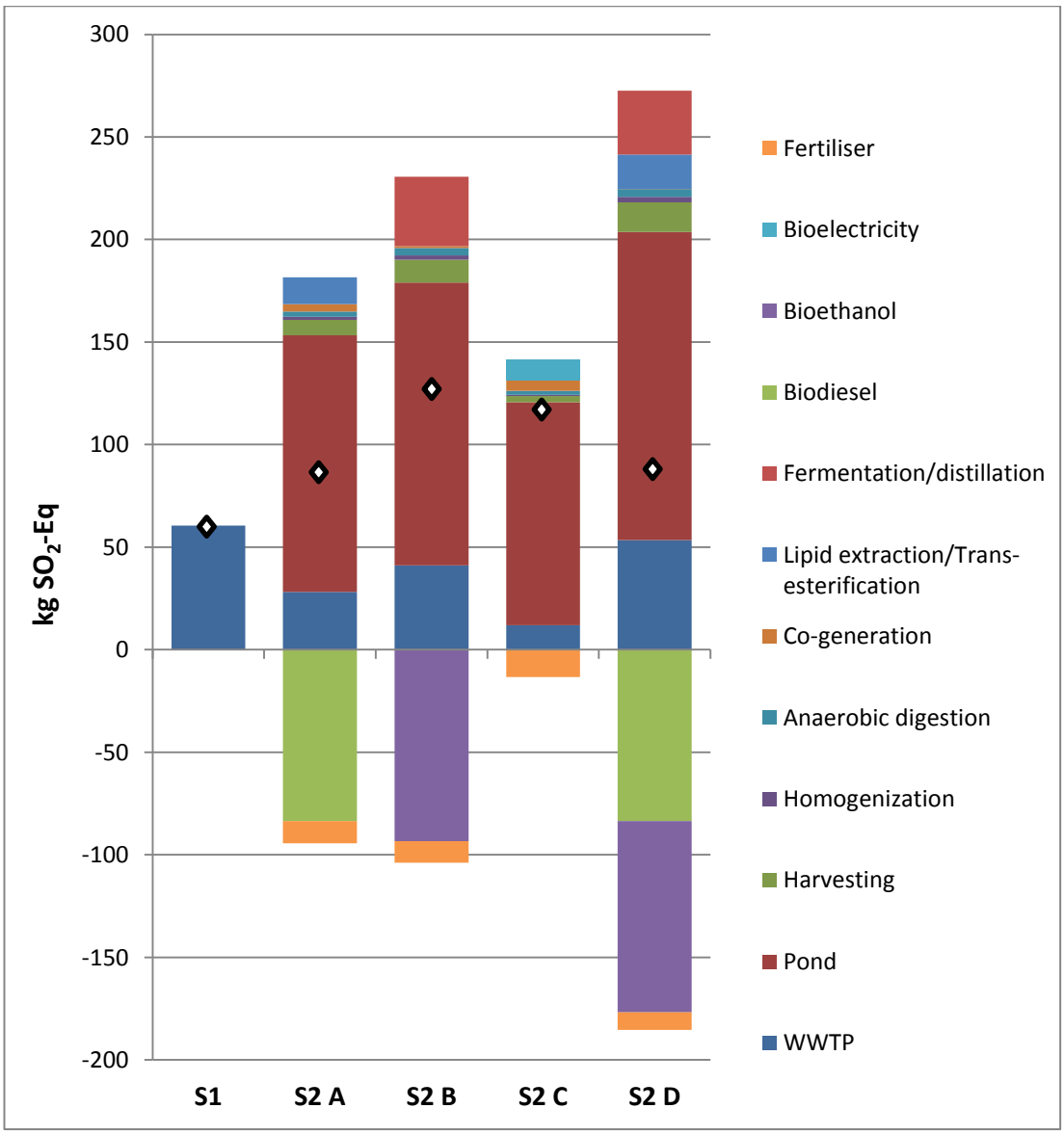


Figure 4-5 The contribution of the unit processes to the acidification potential for each scenario and process stream

(Note: the diamonds represent total net value)

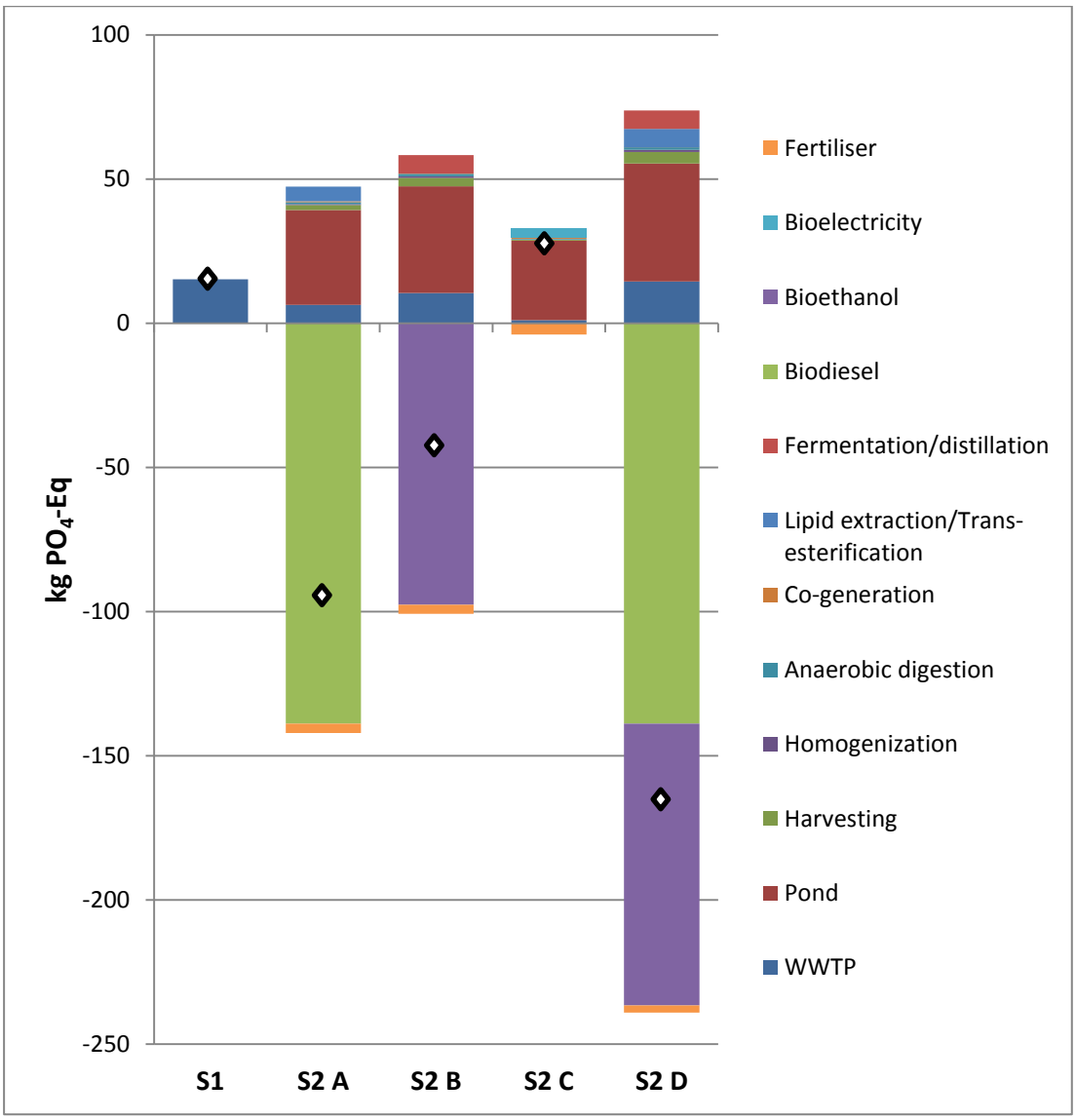


Figure 4-6 The contribution of the unit processes to the eutrophication potential for each scenario and process stream

(Note: the diamonds represent total net value)

4.4.2.1 Global warming potential

Scenario 1 was the only scenario which produced a net positive global warming potential. This scenario was assumed not to produce any product and therefore no global warming potential was offset as a consequence. In the case of scenario 2 and for each process stream, the use of a proportion of the biomass as a source of fertiliser meant that some of the carbon taken up during cultivation was assumed to be sequestered. In each case the carbon uptake was the main source of the negative global warming potential, where the production of biodiesel and bioethanol also offset some CO_2-Eq emissions. S2 C had the greatest negative global warming potential due to the low emissions for energy generation from the biogas produced and the high mass of carbon sequestered as fertiliser. The greatest majority of emissions were from the algal cultivation stage (93.8%). The high concrete use for the algal ponds was the main cause of the high CO_2-Eq emissions accounting for 65.6%. The second highest contributor was the production of superphosphate at 25.7%. The emissions were greater for the other process streams for scenario 2 as a greater proportion of the required energy was sourced from the national grid. The emissions were higher from those processes that required electricity, for S2 A the cultivation stage accounted for 70.1% of emissions. The majority of emissions were a result of the concrete production (39.2%). However the electricity to power the paddlewheel were also high (12.6%). The least amount of biogas was produced in S2 D, the CO_2 emissions for electricity were therefore greatest for this process stream due to the high use of electricity from the national grid. The operation of mechanical mixers for wastewater treatment accounted for 18.6% of the total emissions and the paddle wheels of the cultivation ponds accounted for 17.7%.

4.4.2.2 Acidification

In contrast to the global warming potential, scenario 1 was most favourable in terms of the acidification potential. Each process stream for scenario 2 provided some form of offset through produced products. However the high emissions from each unit process led to high overall emissions. S2 B had the highest emissions mainly as a result of the algal cultivation ponds (59.8%), which similarly to the cumulative energy demand were largely a result of the superphosphate production (32.3%). The

comparatively high use of the national grid (16.8%) for electricity consumption also led to high SO_2-Eq emissions from those processes using electricity. The mechanical aeration for the biological treatment accounted for 14.6% of SO_2-Eq emissions and the paddlewheels in the raceway ponds accounted for 13.9%. Despite high emissions for S2 D, the net SO_2-Eq emission was the lowest because of the high offset due to the production of both biodiesel and bioethanol. The combined production offset 64.9% of the total emissions. S2 A had lower SO_2-Eq emissions than S2 D due to the higher biogas production meaning a lower reliance upon the national electricity grid. However the offset emissions as a result of biodiesel production were not as great as those from both biodiesel and bioethanol production for S2 D.

4.4.2.3 Eutrophication

For eutrophication, most of the scenario 2 process streams outperformed scenario 1 except for S2 C. The negative emissions as a result of the biodiesel and bioethanol production for S2 D far outweighed the greater emissions from each of the unit processes. The total emissions were 73.8 kg SO_2-Eq compared to -239 kg SO_2-Eq offset from the production of biodiesel, bioethanol and biogas. The eutrophication potential was also negative for S2 A and S2 B due to the emission offsets. The emissions were greater for S2 B (58.3 kg SO_2-Eq compared to 47.3 kg SO_2-Eq) because the generated biogas was lower and a higher percentage of the national electricity grid was required. The offset emissions for biodiesel generation were also greater than those for bioethanol generation (-138.8 kg SO_2-Eq compared to 977 kg SO_2-Eq). S2 C produced the least emissions because the electricity exported only offset 10.5% of total emissions produced despite emission being relatively low (29.8 kg SO_2-Eq).

For global warming potential and eutrophication potential, scenario 2 provided the best method for the nutrient removal with a net removal of CO_2 and PO_4 (apart from S2 C). The high carbon sequestration as a result of using the residual algal biomass for fertiliser led to high CO_2 reduction values and negative values for the global warming potential. The low CO_2-Eq emissions and high carbon sequestration from S2 C made this the most favourable process stream when the global warming potential was considered. For S2 D, the high production of biodiesel and bioethanol

led to the greatest negative value of eutrophication potential despite higher process emissions.

For the acidification potential, scenario 1 was the most favourable due to the low emissions from each of the processes. High emissions for each process stream for scenario 2 were a result of the superphosphate required in the algal cultivation process as well as the electricity generation from the national grid which was most applicable to S2 A, B and D. Despite acidification offsets from the products, they did not balance the process emissions.

4.4.3 Sensitivity analysis

In scenario 2 some of the assumptions were adjusted to test the impact upon the results. Research investigating the production of algal bio-energy and more specifically algal bioenergy from biomass produced in a wastewater treatment plant is still currently limited and therefore there are still many uncertainties. The sensitivity analysis focused upon adjusting several assumptions:

1) Reducing/increasing the lipid content by 10%
2) Adjusting the bioethanol conversion rate to a minimum of 5.5% [209] and maximum of 38% [100]
3) Adjusting the methane recovery to a minimum of 0.232 LCH_4/g VS and maximum of 0.310 L CH_4/g VS [17]
4) The wastewater provided sufficient nutrients (no superphosphate required)
5) 50% of waste heat used

Figure 4-5 displays the impacts upon the energy balance and each environmental category for all of the process streams for scenario 2. The figure uses tornado plots which show the base case as the value where the horizontal axis is bisected. For each sensitivity analysis adjustment (1-5), the corresponding result is displayed either as a greater or lower value than the base case. For adjustments 1-3, the red bar represents the upper value used and the green bar represents the lower value used.

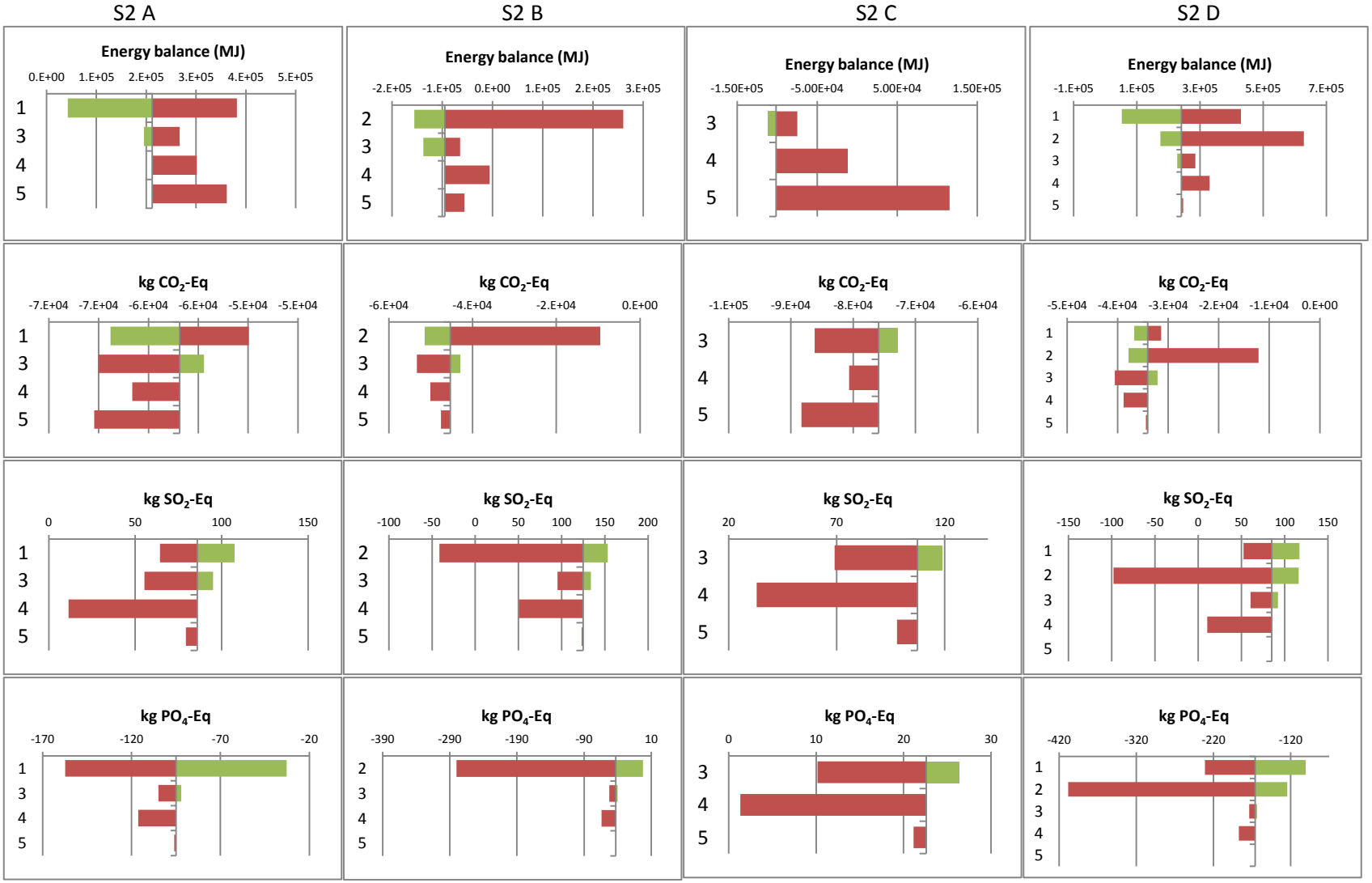


Figure 4-7 Plots displaying the impact of each sensitivity analysis adjustment on the energy balance and each environmental impact category for all process streams of scenario 2

The variation of the lipid concentration in the biomass had a large impact upon the energy balance of S2 A and S2 D. An increase in the lipid content increased the energy balance of S2 A to 381,843 MJ, an 80.0% increase over the base case. A similar decrease in the lipid content reduced the energy balance to 42,404 MJ, 20.0% of the base case. Similar results were recorded for S2 D, a maximum energy balance of 429,925 MJ was calculated alongside a minimum of 52,000 MJ. The increase in lipid content increased the global warming potential for both S2 A and S2 D because less fertiliser was produced and consequently less carbon was sequestered. In contrast, with a lower lipid content, the global warming potential was more negative for both process streams, a 12.2% increase in negativity for S2 A and an 8% increase for S2 D. Increasing the lipid content had a very positive impact upon the acidification and eutrophication potentials, particularly eutrophication where a 65.5% increase in PO_4 savings were recorded for S2 A.

The increase in the conversion rate of biomass to bioethanol had a substantial impact upon the energy balance and each impact category. For S2 B, the increase in the conversion rate increased the energy balance from -94,776 MJ to 260,403 MJ, a greater value than the base case for S2 A. The lower conversion rate reduced the energy balance to -180,740 MJ. Similarly to the change in lipid content, a higher conversion rate to bioethanol had a negative impact upon the global warming potential despite greater offsets from the increased bioethanol production. The global warming potential increased to -9,476 kg CO_2-Eq from -45,323 kg CO_2-Eq. In contrast, the global warming potential negatively increased when the conversion rate was lower, the results were similar for S2 D. Predictably for the acidification and eutrophication potentials the higher conversion rate greatly reduced the acidification potential for S2 A from 125 kg SO_2-Eq to -41 kg SO_2-Eq as a result of the offsets from bioethanol production. For the increased value of conversion rate a similar reduction for the eutrophication was recorded from -43 kg PO_4-Eq to -280 kg PO_4-Eq. For S2 D, a maximum negative eutrophication potential of -408 kg PO_4-Eq was recorded.

Adjusting the methane conversion rate had an impact upon all of the process streams as biogas was assumed to be generated in each case. The increase in

methane conversion had a positive impact upon the energy balance and each environmental impact for all of the process streams although the increases were less than the increased lipid content and ethanol conversion rate. The greatest positive impact in terms of the energy balance was for S2 B, where the negative energy balance reduced by 57.4%, the value was still however negative. Despite an improvement to the energy balance of S2 C, the value also remained negative. The increased methane production had a highly positive impact upon the global warming potential as more electricity was produced to supply processes, therefore reducing dependency upon the national grid which has a high global warming potential. The greatest negative increase was for S2 D with an increase of 19.0%. The reduced values for eutrophication were similar for each process stream, the greatest reduction was for S2 C with a reduction of 35.7% and the least reduction was for S2 B at 23.9%. The values for the eutrophication potential avoidance increased for S2 A, S2 B and S2 C by 10.4%, 22.6% and 4.7% respectively. For S2 C the eutrophication emissions remained positive but reduced by 55.1% assuming a greater methane yield.

When the use of superphosphate was not included, the change to each of the impacts was considerable. For S2 A and S2 D the energy balance increased by 42.1% and 37.1% respectively. The energy balance values became less negative for S2 B and S2 C by 94.2% and 88.2% respectively. The avoidance of superphosphate had a comparatively small impact upon the global warming potential, the greatest increase in avoided emissions was for S2 D where an increase of 13.9% was recorded. Substantial savings were made to the acidification potential by removing the superphosphate input. The greatest acidification reduction was for S2 C with a reduction value of 87.9%. The lowest reduction was for S2 B at 59.6%. Similarly, considerable large benefits were recorded for the eutrophication potential with an increase in emission avoidance of 49.6% for S2 B and reduction in emission of 94.1% for S2 C.

When it was assumed that 50% of heat generated through co-generation of the biogas was used to replace heat from natural gas, the increase in the energy balance for S2 A, S2 B and S2 C was high. The energy balance for S2 A increased

from 212,149 MJ to 362,024 MJ, a 70.6% increase. A 58.6% increase was recorded for S2 B and the value for S2 C changed from -101,215 MJ to 115,198 MJ. The increase for S2 D was much lower at 2.3%.

The use of the heat also had a beneficial impact for each of the environmental impacts although the benefit was far lower than for the energy balance. The global warming potential avoided increased by 16.3% for S2 C and 15.1% for S2 A. The acidification potential reduced by 7.6% for S2 A and 8.9% for S2 C, again little difference was recorded for S2 D. The eutrophication values were largely unaffected although a reduction of 6.7% was recorded for S2 C.

Table 4-8 displays the maximum and minimum value of the energy balance and each environmental impact and the process stream and input adjustment.

Table 4-8 Maximum and minimum values of the energy balance and environmental impacts for each process stream and input adjustment

Category	Value	Process stream	Input adjustment
Max energy balance	429,925 MJ	S2 D	High lipid content
Min energy balance	-15,748 MJ	S2 B	Low bioethanol conversion
Low GWP	-86,174 kg CO_2 kg-Eq	S2 C	High methane conversion
High GWP	-4,976 kg CO_2-Eq	S2 B	High bioethanol conversion
Low acidification	-98 kg SO_2-Eq	S2 D	High bioethanol conversion
High acidification	154 kg SO_2-Eq	S2 B	Low bioethanol conversion
Low eutrophication	-408 kg PO_4-Eq	S2 D	High bioethanol conversion
High eutrophication	26 kg PO_4-Eq	S2 C	Low methane conversion

Supposing the algal biomass that is cultivated in the algal treatment ponds has a lipid content as high as 30% or can be manipulated through low nitrogen conditions the results of the sensitivity analysis suggest that a highly positive energy balance is possible. Despite the assumption that the species which are likely to dominate the ponds would not have a particularly high lipid content it has

been proven that under nitrogen limiting conditions the lipid content increases [29]. This technique could potentially be used following the nutrient uptake to raise the lipid content and provide higher energy yields.

The greatest global warming potential saving was calculated for S2 C where a high conversion of biomass to methane was assumed. A high proportion of the biomass was determined to be used as fertiliser and therefore a high degree of carbon sequestration was calculated. Additionally as an excess of electricity was generated this was assumed to offset electricity produced by the national grid which provided high CO_2-Eq savings.

The most preferable results for acidification were for S2 D when the bioethanol conversion was assumed to be high. The acidification value offset by the high yield of bioethanol alongside the production of biodiesel led to a highly negative value. For similar reasons the eutrophication value was also the most preferable for S2 D when the bioethanol conversion was high.

S2 B provided the worst results for the energy balance, global warming potential and acidification. For the energy balance and acidification the results were least favourable when a low bioethanol yield was assumed. This is due to low energy yield and high processing requirements. The comparatively low GWP saving was calculated when a high bioethanol conversion rate was assumed and was a result of only a small proportion of the biomass being used for fertiliser.

The highest eutrophication was recorded for S2 C when a low conversion rate to methane was used. As no energy was exported there was no eutrophication offset and the lower methane yield led to a higher use of electricity from the national grid, raising emissions.

4.5 General discussion

The aim of this study was to investigate whether it was more sustainable to upgrade Haifa WWTP for nutrient removal using conventional methods (scenario 1) or to use novel method employing the cultivation of algal biomass (scenario 2). The conventional method assumed the use of the A_2O process for nutrient removal and the novel method assumed the cultivation of algal biomass in wastewater following prior treatment with mechanical aeration, the algal biomass was harvested and processed to recover energy from a variety of potential streams. The sustainability was determined based on the energy balance (the energy produced minus the energy required), the global warming potential, the acidification potential and the eutrophication potential.

In terms of the energy balance, the conventional method of nutrient removal, scenario 1, produced a net negative value of -80,487 MJ. This value was higher than both S2 B and S2 C. S2 A and S2 D produced positive values with S2 D being marginally higher at 240,960 MJ. The high energy recovery value of biodiesel was one reason for the high values of energy balance. Additionally, the high mass of residual biomass available for anaerobic digestion allowed much of the energy to be provided for by the produced biogas. The base case relied upon a relatively high assumed content of lipids in the biomass (20%). When a lower lipid content of 10% was assumed considerably lower energy balance values of 42,404 MJ and 52,000 MJ were recorded for S2 A and S2 D respectively. The values were however positive and remained greater than the base case values for S1, S2 B and S2 C. These results had similarities to those produced by Sturm *et al.* [204] where the authors investigated the recovery of biodiesel from wastewater grown algae. An energy recovery (as biodiesel) of 4,500 kWh/d (16,560 MJ/d) was recorded with an energy consumption of 2,800 kWh/d (10,080 MJ/d) producing an energy balance of 1,700 kWh/d (6,120 MJ/d). The study was for a lower flow of wastewater (12 Mgd or 54,553 m^3/day) and assumed a lower biomass productivity (12 g/m^2/day), lipid content (10%) and did not include anaerobic digestion of the residual biomass. When the lipid content assumed in this study was 10%, a more similar energy balance of 42,404 MJ was calculated. The LCA study conducted by

Clarens *et al.* [27] also produced a positive energy balance when cultivation was assumed to use previously treated wastewater, an energy demand of 290 GJ was determined for a production of 317 GJ of biomass derived energy.

Increasing the conversion rate of biomass to bioethanol to the maximum value (38%) had a considerable impact upon the energy balance, providing a value of 628,892 MJ for S2 D. The probability of obtaining such a high conversion rate is likely to be low. Most studies have reported yields that are far lower [101, 172, 174]. When the minimum conversion rate of 5.5% was assumed an energy balance more negative than S1 was reported for S2 B. The results suggest bioethanol production should not be considered unless high conversion rates can be proven. Both the avoidance of using superphosphate and making use of 50% of the waste heat greatly increased the energy balance of all process streams and the potential of both of these should be considered as a method of enhancing the sustainability of the system.

Each of the process streams for scenario 2 provided better results for global warming potential than for scenario 1. The reason for this was the uptake of CO_2 during the cultivation process and subsequent sequestration as carbon in the residual biomass used as fertiliser. Where biodiesel and bioethanol were produced, the avoided emissions also benefitted the global warming potential although the greatest negative global warming potential was for S2 C because a greater proportion of the biomass was converted to fertiliser. For this reason S2 C provided the best process stream for global warming potential and can be considered a good method for carbon sequestration. The net uptake of CO_2-Eq contrasted with other studies where bioenergy from algae was considered, Lardon *et al.* [35] calculated a net positive global warming potential due to processing and combustion emissions. Their study assumed the use of fertiliser and included emissions related to combustion. Similarly, Clarens *et al.* [27] calculated net positive emissions as a result of high processing emissions. Net negative emissions were, however recorded when source separated urine was assumed to be used as the growth media.

A greater assumed lipid content and a maximum bioethanol conversion increased the global warming potential as less biomass was calculated to be used as fertiliser and therefore less CO_2 was sequestered. Conversely, with a lower lipid content and minimum bioethanol conversion the global warming potential increased negatively, sequestering a greater amount of carbon however energy yields were reduced. Higher and lower assumed methane yield led to greater and lower CO_2 sequestration respectively. If a high methane yield was possible, a greater amount of electricity could be exported and offset electricity produced for the national grid. This scenario for S2 C produced the greatest potential for CO_2 sequestration. Avoiding superphosphate use reduced CO_2-Eq emissions, as did the use of heat but by a lesser amount.

In contrast to the global warming potential, scenario 1 produced the best results for the acidification potential. The high acidification potential associated with superphosphate and concrete production led to high emissions from all of the process streams for scenario 2 despite the avoidance of acidification emissions through the production of biodiesel and bioethanol in some cases. Many of the adjustments in the sensitivity analysis made a large impact upon the acidification potential. The greatest impact was a result of increasing the bioethanol conversion rate where negative acidification values were calculated suggesting a strong improvement over S1. Avoiding the use of superphosphate also greatly reduced the acidification potential and led to all of the process streams comparing favourably to S1.

For the eutrophication potential, most of the process streams for scenario 2 provided a negative eutrophication potential value as a result of the biodiesel and bioethanol produced and therefore compared well with S1. S2 C produced the highest eutrophication potential value largely as a result of the superphosphate production and lack of offset emissions from exported energy. The changes to the bioenergy yields had the greatest impact to the eutrophication potential for the sensitivity analysis particularly for higher bioethanol conversion rates where a very high eutrophication offset was recorded for S2 D. The avoidance of

superphosphate also reduced greatly the eutrophication potential however not by the same extent.

The results suggested that the more sustainable method to upgrade the Haifa WWTP would be to use algal biomass cultivation. The results, however, were very dependent upon the energy recovery processes employed. The production of biodiesel and biogas (S2 A) produced a high energy balance. The production of biodiesel, bioethanol and biogas (S2 D) from the residual biomas appeared more favourable. It should be noted however that if the lipid content was low at 10%, the energy balance values were only narrowly positive but still greater than S2 A. If a high bioethanol recovery is possible (38% recovery), the S2 D process stream would be by far the most sustainable method of upgrading the wastewater treatment plant in terms of the energy balance as well as the acidification and eutrophication potentials. This value for bioethanol recovery has however only been achieved for a specific strain of algae under certain conditions and therefore such a high recovery efficiency may be unlikely. Due to the relatively low energy yield as electricity generated from produced biogas, producing only biogas appear one of the least sustainable choices although if the heat can be used and high methane yields are possible then the process by itself is potentially worth considering. In each case avoiding the use of superphosphate greatly increased the energy balance and had a positive impact upon the environmental impacts. Due to the characteristics of the wastewater however, phosphate is likely to be the limiting nutrient and some form of supplementation may be required to allow continued growth. The results however show the negative impact of superphosphate use and therefore its use should be avoided where possible.

Despite scenario 2 appearing most promising there are several key assumptions that may jeopardise the systems viability. One assumption is the high year round productivity of the algae which given the varying climatic conditions between seasons is highly unlikely. Most studies around the world report substantial differences in productivity between seasons [210] and this is also the case in Israel [47]. It's highly likely that the productivity in winter would decrease thereby reducing the effectiveness of the nutrient removal and potential energy recovery. It

is unlikely that this difficulty could be avoided as artificially heating and lighting the ponds would most likely be unviable. In this case when productivities are too low in winter an alternative treatment method would be necessary involving a similar set-up to scenario 1. This however would greatly reduce the sustainability of the treatment method due to higher infrastructure requirements and a lower annual bioenergy yield. On the other hand, the productivity in the summer months may be greater which would allow a lower hydraulic retention time and a higher overall energy balance due to lower energy inputs.

Another key assumption is that the biomass will not be affected by predatory bacteria and other plant types. This is another problem that could have a strongly negative impact upon the productivity of the biomass which would reduce the effectiveness of nutrient removal and the recovery of energy. Avoiding contamination is difficult with open ponds. The use of insecticide could potentially provide a solution however insecticides have been shown to have a high environmental and health impact [211, 212] which may reduce the overall system sustainability.

The high areal requirement for nutrient removal using algal biomass means the land cannot be used for alternative uses such as agriculture. In the case of Haifa wastewater treatment plant the surrounding area is used for agriculture. If scenario 2 was to be implement much of this land would be required for the cultivation ponds, therefore reducing land for food production. This is potentially a highly negative impact considering the food versus fuel debate [213]. The benefit, however, is the production of fertiliser which can be used on the remaining agricultural land. In the cases of other wastewater treatment plants it is possible that insufficient land area would be available which may be a limiting factor.

This study suggests that the use of algal biomass cultivation can be a more sustainable method of removing excess nutrients for the particular case of Haifa WWTP. The study however relied upon key assumptions which due to limited research have not been proven on a large scale. The results suggested that the recovery of biodiesel, bioethanol and biogas together from the biomass would provide the greatest energy balance and was also more favourable in terms of

acidification and eutrophication but not global warming potential. The sensitivity analysis showed that the results were highly sensitive to adjustment of the oil content as well as bioethanol and methane conversion rates. A lower oil content could greatly reduce the energy balance as could lower conversion rates to bioethanol and methane. In contrast higher values led to much higher with more sustainable values being recorded.

Further research needs to be conducted to consider the possibility of cultivation algal biomass in tertiary wastewater to investigate the year round productivity rates, nutrient uptake, species dominance, harvestability and energy recovery yields. Providing that the assumptions made in this study are proven to be relatively accurate, algal cultivation for nutrient recovery in Haifa WWTP could be the most sustainable option.

4.6 Conclusions

Recently there has been an interest in the concept of incorporating algal cultivation with wastewater treatment to reduce the inputs such as fertilisers for the cultivation process. Few studies have looked at how the sustainability of such a system would compare to a conventional method of treatment. This study compared the sustainability of using algal cultivation as a method of upgrading a wastewater treatment plant for nutrient removal in Israel compared to a more conventional method. The study considered the energy balance and several environmental impacts of both methods by examining the material and energy usage as well as the value of the products produced. The results suggested that in terms of energy balance, global warming potential and eutrophication potential the cultivation of algal biomass in the effluent would be more sustainable providing the maximum amount of energy is recovered from the biomass as biodiesel, bioethanol and biogas. If a high lipid content (30%) could be stimulated in the biomass, this would greatly improve the energy balance by 78.4%. Any manipulation of the biomass may, however, prove difficult under environmental conditions. Further work through pilot scale set-ups is necessary to test the feasibility of the system and the assumptions made.

5 A life cycle assessment of macro-algae (seaweed) cultivation and processing for biofuel production

5.1 Introduction

As mentioned in previous chapters, the majority of research for algal bioenergy has been focussed upon microalgae and biodiesel production. Macroalgae, which is prevalent in most coastal regions of the world, however, is also being considered as a potential energy feedstock. The marine cultivation of seaweed does not generally require arable land or fertiliser where the necessary elements for growth are typically found in the coastal environment [42]. Also in comparison to most terrestrial crops the biomass yield of macro-algae over a growing season is higher [157]. Nevertheless, there are few systems which are currently operating to cultivate and obtain energy from macro-algal biomass on a large scale and the sustainability of such a system is largely untested [19]. Research has been conducted in a number of studies dating back to the 1980s to investigate the potential yields of energy recovery from macro-algal biomass [122, 127]. These studies have mainly been concerned with the production of methane [122]. More recently attention has been paid to the recovery of bio-ethanol [115]. High yields of both methane [214] and bioethanol [115] have been recorded but consequent scaling up has been limited. As a result the overall sustainability of such systems are largely unknown. Recently, however, there have been several life cycle assessment (LCA) studies conducted which have examined the sustainability of resource and energy recovery from brown seaweed [42, 215]. Langlois *et al.* [42] focussed upon the environmental impacts of the generation of methane from brown seaweed (*Saccharina latissima*) from the whole seaweed and following alginate production. Their results indicated that compared with natural gas the impacts were less favourable although improvements to the design allowed both processes to compare more favourably. Alvarado-Morales *et al.* [215] investigated two production scenarios from brown seaweed (*Laminaria digitata*) in Nordic conditions; one producing just biogas and the other, bioethanol with the stillage being converted to biogas. The authors compared the energy process balance of the two scenarios and the potential environmental impact on global warming, acidification and terrestrial eutrophication. The production of both

bioethanol and biogas facilitated a greater energy export however the environmental impacts were poorer as a result of the extra fermentation and distillation process. These studies suggest bioenergy generation from macroalgae is a promising concept although the focus has been largely on brown seaweed for large scale off-shore sites.

Therefore, the aim of this study was to use a life cycle assessment approach to investigate the sustainability of producing bioenergy from macroalgae using local cultivation techniques and species that are common to a given area considering the production of bioethanol, biogas or both. It should be noted due to the relatively new state of research, there are few studies which provide values that could definitely be replicated on a large scale. Values used in this study were therefore taken from a variety of studies, and chosen because they were deemed the most suitable.

5.2 Materials and Methods

5.2.1 The case study

Chile has an extensive coastline and much of the coastal area is suitable for the growth of seaweed. In the past, Chile has been one of the largest producers of alginate, agar and carrageenan, all of which are derived from macroalgae [216], traditionally using wild stocks. Overexploitation, however, has made cultivation necessary [151]. In Chile, macroalgae cultivation has largely been restricted to red algae and more specifically *Gracilaria chilensis,* due to the relatively simple farming methods that have been developed [151]. Brown macroalgae, particularly *Macrocystis pyrifera* or giant kelp, is also becoming an important resource and alongside *G. Chilensis*, is one of the favoured species for cultivation due to its economic importance (abalone (*Haliotidae*) farming, fertiliser) and potentially high yields [66]. Cultivation of *G. chilensis* is commonly conducted by 'bottom planting' where thalli of previously cultivated biomass is planted into the seabed sub-tidally and provides several harvests before requiring replanting [60]. 'Long-line cultivation' by tying thalli to ropes and deploying the ropes off-shore has also been successfully demonstrated and can offer cultivation on a larger scale [66] .Long-line cultivation of *M. pyrifera* is the traditional method of culture however lines are first inoculated with spores prior to subsequent deployment offshore. This method can typically deliver very high yields of biomass on a large scale [217].

5.2.2 Goal and Scope

The goal of this LCA study was to determine the most sustainable method of cultivating and processing macroalgae to bioenergy in Chile. For the purpose of this study, the three above-mentioned cultivation scenarios of macro-algae as potential sources of bioenergy were modelled. These included: bottom planted *G. chilensis* (S1), long line cultivated *G. chilensis* (S2), and long line cultivated *M. pyrifera* (S3). Furthermore, following cultivation, the conversion of the biomass to bioenergy was considered. Three different bioenergy pathways were modelled: fermentation and distillation to bioethanol (P1), anaerobic digestion to biogas and subsequent conversion to electricity (P2) or both, with the stillage produced from the bioethanol plant being anaerobically digested and the biogas converted to electricity (P3). Nine different process streams were therefore modelled in total.

The sustainability metrics chosen were the Energy Return on Investment (EROI) and six environmental impact categories. For each environmental impact, all of the contributing emissions are calculated as one emission based on their equivalent impact (i.e., climate change – GWP 100a (kg CO_2-Eq); acidification potential – generic (kg SO_2-Eq); eutrophication potential – generic (kg PO_4-Eq); stratospheric ozone depletion – ODP steady state (kg CFC-11-Eq); photochemical oxidation – High NO_x POCP (kg ethylene-Eq); human toxicity – HTP 100a (kg 1,4-DCB-Eq)).

The EROI is defined very generally as the ratio of the output energy of a system to the input energy to the system [218]. In this study, the EROI was calculated as the ratio of the lower heating value of energy carriers to the total cumulative non-renewable fossil energy demand (Eq. 5-1).

$$EROI = \frac{Total\ energy\ produced\ (lower\ heating\ value)}{Total\ cumulative\ non{-}renewable\ energy\ demand} \quad \text{(Eq. 5-1)}$$

Alongside the EROI analysis, the six environmental impact categories provided a wide scope for comparison. The impacts were determined by combining the emissions from each unit process and calculating the contribution to each impact category using the Centrum voor Millikunde Leiden method (CML 2001). The results of each scenario were compared to the same metrics for energy in the form of

petroleum, bioethanol from corn and electricity from biogas obtained from a mix of biowaste and sewage sludge, all of which were calculated using data from Ecovinvent [106].

The functional unit used for comparison was 1 MJ of energy, as the lower heating value of the energy produced. The inputs to the inventory were detailed for one hectare of cultivation area for clarity. For each scenario, inputs to model a base case were used. As part of the sensitivity analysis several inputs were adjusted to more favourable values to investigate the impact upon the results of the model. The study considered the cultivation of species that are common to Chile but which can also be found in comparable environments: the results are therefore also applicable to these environments.

5.2.3 System model

5.2.3.1 Cultivation

Three cultivation scenarios (S1-S3) were modelled (Fig. 5-1). Scenario 1 (S1) was the bottom planting of *G. chilensis*. The first step of the process stream was preparation of the thalli followed by planting of the thalli 1 km from the landing point and processing facilities. The biomass was then assumed to be harvested by a diver and fishing vessel. The total cultivation area was considered to be 20 ha. Scenario 2 (S2), the long-line cultivation of *G. chilensis*, was initiated by tying previously cultivated biomass thalli to ropes. The ropes were then deployed 10 km from the landing point using a barge vessel. Following a culture period of 6 months they were assumed to be harvested by the same vessel. The total cultivation area was considered to be 100 ha, an area greater than bottom cultivation because there is no depth limit as there is with bottom cultivation. Scenario 3 (S3), the long-line cultivated culture of *M. pyrifera*, required first the inoculation of the lines with spores and subsequent development in tanks as part of the hatchery process. The lines were then assumed to be transported 10 km from the landing point to the cultivation site by barge and harvested by the barge at the end of the 9 month culture period. As with S2, the area of cultivation in one development was assumed to be 100 ha.

5.2.3.2 Processing

The processing of the biomass was common to each scenario and was assumed to be conducted beside the landing site. Pre-processing of the biomass included the transport of the biomass from the landing site on a conveyor belt and grinding using a wet biomass attritor. The biomass was then either processed using one of the three production alternatives, P1, P2 or P3. P1, the generation of bioethanol was modelled using data from Ecoinvent for the production of bioethanol from corn [219] as data for macroalgal biomass is currently lacking. The model includes data for pre-treatment, saccharification, fermentation and distillation. P2, the generation of biogas was modelled using the method for anaerobic digestion of sludge in wastewater treatment [200] considering the mixing and heating of the biomass. P3, the generation of bioethanol followed by the processing of the residual biomass to biogas using anaerobic digestion was modelled using the same methods employed for P1 and P2, respectively.

5.2.3.3 Products

The high polysaccharide and low oil content of macroalgae favours the production of bioethanol and biogas [19], therefore these were the two products considered in this study. Using assumptions consistent with Ecoinvent [219], the bioethanol was assumed to be upgraded to 99.7% ethanol and biogas (63% CH_4) was assumed to be converted to electricity and heat in a biogas co-generation engine. A proportion of the electricity and heat produced from the biogas was assumed to be used as the energy source for the system (the proportion was dependent upon the scenario). The remaining electricity was exported to the national grid as the produced energy and the heat was not used, although the environmental impacts of the heat produced were allocated to the exported electricity. The lower heating value of bioethanol (28.1 MJ/kg) [219] and the energy value of electricity produced from the biogas (as the lower heating value) were used for comparison. Following the generation of either or both bioethanol and biogas, the stillage and excess digestate were assumed to be used as fertiliser offsetting the impacts of the production of conventional fertilisers (i.e., ammonium sulphate, superphosphate and potassium chloride). The values were based on the nutrient contents of the biomass and their bioavailability (See Appendix

C.2). As the produced fertiliser contains a proportion of the total carbon contained within the cultivated seaweed this was considered as the mass of carbon extracted from the atmosphere during the cultivation process. The calculated value was negated against the CO_2-Eq emissions of the system to determine the global warming potential (See Appendix C.2).

5.2.3.4 Energy supply

Where biogas was produced, a proportion of the electricity and heat was assumed to provide the necessary energy for each process as required. The electricity and heat generated from the biogas was calculated using data from the Ecoinvent inventories for bioenergy [219] where it was detailed that for every 1 MJ of biogas (63% CH_4 content, and lower heating value of biogas of 22.73 MJ/m^3) produces 0.55 MJ of heat and 0.32 MJ of electricity. The environmental impacts were allocated based on exergy values of 1 for electricity and 0.17 for heat [219]. The impacts were allocated to the processes which use the energy based on the proportion of use. The impacts of the co-generation of biogas converted to electricity for export were allocated to "biogas cogeneration (export)" which included the impacts allocated to the waste heat.

Where biogas was not produced or insufficient biogas was produced to supply enough energy, the required electricity was assumed to be provided by the Chilean national grid which was modelled on available data (Appendix C.2). Heat was assumed to be provided by a natural gas boiler which was modelled on the data for heat, natural gas, at boiler condensing modulating >100kW from the Ecoinvent database [106].

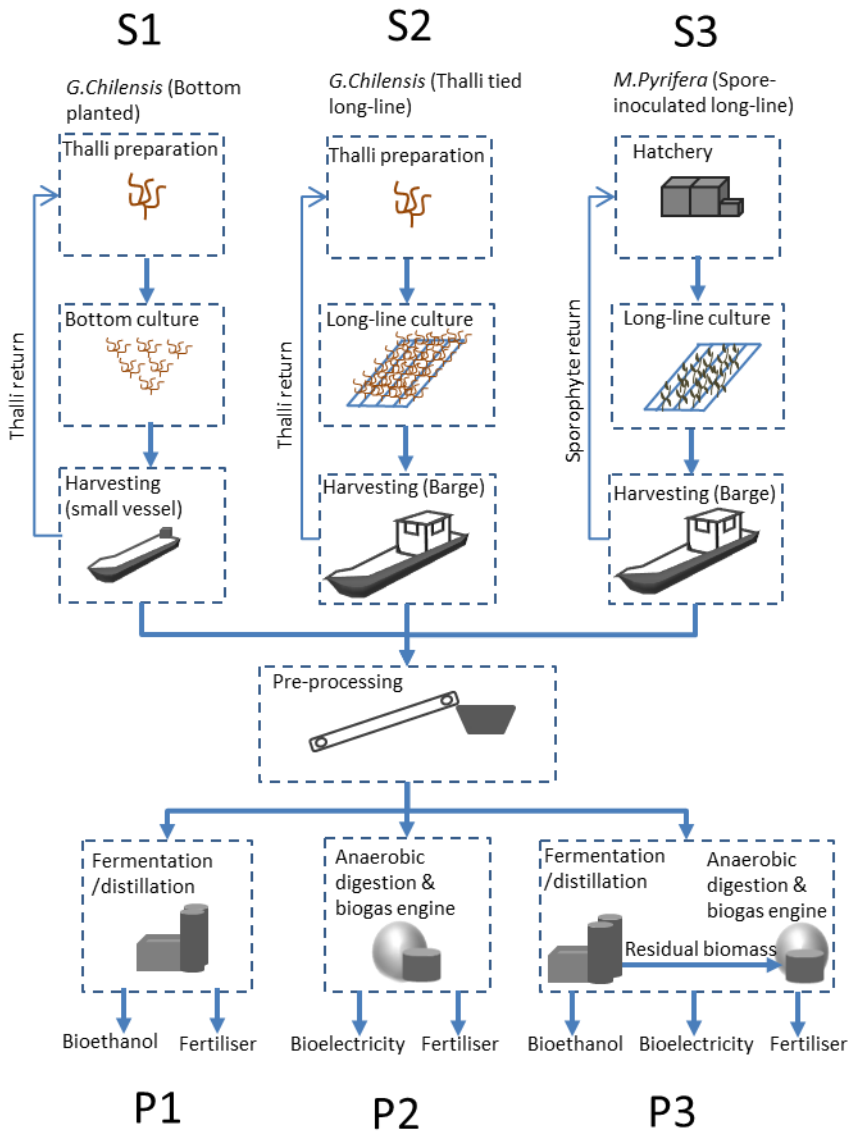


Figure 5-1 Flow diagram with each unit process considered in the LCA for the three cultivation scenarios and three processing streams

5.2.4 Data acquisition and modelling

Data was acquired from a variety of sources but mainly from published literature and personal communication with seaweed farmers during 2012. The data for material and energy use necessary to calculate the cumulative energy demand and environmental impacts for each unit process were obtained from the Ecoinvent database [106] and were calculated using OpenLCA [206] and compiled in Microsoft Excel.

5.2.5 Life cycle inventory

The assumptions of each scenario related to the biomass characteristics, productivity rates and bioenergy yields are detailed in Table 5-1. The inputs to the unit process and methods of attainment are provided in Tables 5-2 and 5-3 (For detailed information see Supplementary data A).

Table 5-1 Input values assumed for the productivity, characteristics and biofuel yields from each scenario

	S1		S2		S3	
	Value	Ref.	Value	Ref.	Value	Ref.
Productivity (t/ha/y) (d.w.)						
Lower	12.6	[60]	7.6	[65]	16.5	[62]
Upper	19.2	[60]	12.6	[66]	18.8	[66]
Base case	15.9	-	10.1	-	17.6	-
Characteristics						
Carbon content (%)	30.0	[78]	30.0	[78]	30.0	[78]
VS content (%)	58.9	[127]	58.9	[127]	58.9	[63]
N content (%)	2.80[a]	[155]	2.80[a]	[155]	1.90	[63]
P content (%)	0.96[a]	[155]	0.96[a]	[155]	0.33	[63]
K content (%)	11.40[b]	[220]	11.40[b]	[220]	9.34	[221]
Bioethanol production						
Lower yield (kg/kg biomass)	0.038	[117]	0.038	[117]	0.109	[222]
Upper yield (kg/kg biomass)	0.079	[116]	0.079	[116]	0.132	[64]
Base case (kg/kg biomass)	0.059	-	0.059	-	0.120	-
Biogas production						
Lower yield (L CH_4/g VS)	0.18[c]	[126]	0.18[c]	[126]	0.20[e]	[223]
Upper yield (L CH_4/g VS)	0.23[d]	[127]	0.23[d]	[127]	0.41[f]	[214]
Base case (L CH_4/g VS)	0.21	-	0.21	-	0.30	-

(Note: [a] Calculated using mean value of N and P content and molecular weight of N and P. [b] Value for *Gracilaria salicornia.* [c] Retention time of 82 days and temperature of 37°C. [d] Retention time of 58 days and temperature of 32°C. [e] Retention time of 37 days and temperature of 37°C and assumes a biogas methane content of 65%. [f] Retention time of 46 days and temperature of 35°C)

Table 5-2 Life cycle inventory inputs for each scenario

S1	S2	S3
Preparation	Preparation	Hatchery
Shed: 0.67m^2 Lighting: 5.6 kWh	Shed: 1.33 m^2 Lighting: 22.4 kWh Polyamide rope: 111.9 kg	Shed: 0.67 m^2 Lamps: 960 kWh Pumping: 0.62 kWh Aeration: 8.05 kWh Water treatment: 3.02 kWh Ammonium nitrate, as N: 0.25 kg Sodium phosphate: 0.38 kg Polyamide rope: 50.4 kg
Cultivation	Cultivation	Cultivation
Diesel: 23.7 kg Steel: 0.71 kg Aluminium: 0.98 kg	Polyamide rope: 21.5 kg Concrete: 952 kg Steel: 38.4 kg Polyethylene: 52.5 kg Diesel (Barge): 0.045 kg Barge: 1.67×10^{-4} Diesel (observation): 0.68 kg Aluminium: 0.36 kg	Polyamide rope: 111.0 kg Concrete: 952 kg Steel: 38.4 kg Polyethylene: 52.5 kg Diesel (Barge): 0.072 kg Barge: 1.67×10^{-4} Diesel (observation): 1.02 kg Aluminium: 0.36 kg
Harvesting	Harvesting	Harvesting
Diesel: 71.0 kg Steel: 4.94 kg Aluminium: 6.83 kg	Diesel: 4.78 kg Barge[1]: 1.67×10^{-4}	Diesel: 8.35 kg Barge[1]: 1.67×10^{-4}
Pre-processing	Pre-processing	Pre-processing
Conveyor belt Electricity: 5.51 kWh Steel: 2.16 kg Rubber: 3.45 kg Attritor Electricity: 289.3 kWh Steel: 22.5 kg	Conveyor belt Electricity: 3.58 kWh Steel: 0.43 kg Rubber: 0.69 kg Attritor Electricity: 188.0 kWh Steel: 4.49 kg	Conveyor belt Electricity: 6.38 kWh Steel: 0.43 kg Rubber: 0.69 kg Attritor Electricity: 334.9 kWh Steel: 4.49 kg

(Note: [1]The fraction of a barge tanker)

Table 5-3 Life cycle inventory inputs for each process stream

P1	**P1**	**P1**
Electricity: 515.1 kWh Heat: 14,601.0 MJ Ammonium sulphate, as N: 36.5 kg Diammonium phosphate, as N: 36.5 kg Soda powder: 136.2 kg Sulphuric acid: 90.8 kg Ethanol fermentation plant[1]: 7.99×10^{-6}	Electricity: 334.7 kWh Heat: 9,487.7 MJ Ammonium sulphate, as N: 23.7 kg Diammonium phosphate, as N: 23.7 kg Soda powder: 88.5 kg Sulphuric acid: 59.0 kg Ethanol fermentation plant[1] : 5.19×10^{-6}	Electricity: 606.0 kWh Heat: 17,999.5 MJ Ammonium sulphate, as N: 42.2 kg Diammonium phosphate, as N: 42.2 kg Soda powder: 157.6 kg Sulphuric acid: 105.1 kg Ethanol fermentation plant[1]: 9.31×10^{-6}
P2	**P2**	**P2**
Electricity: 601.7 kWh Heat: 14,783.0 Concrete: 2,776.6 kg	Electricity: 391.0 kWh Heat: 11,318.8 MJ Concrete: 2,184.4	Electricity: 696.5 kWh Heat: 16,342.2 MJ Concrete: 3,043.2 kg
P3 (+P1 inputs)	**P3 (+P1 inputs)**	**P3 (+P1 inputs)**
Electricity: 530.8 kWh Heat: 13,617.1 MJ Concrete: 2,577.3 kg	Electricity: 344.9 kWh Heat: 10,561.2 MJ Concrete: 2,054.9 kg	Electricity: 527.6 kWh Heat: 13,564.7 MJ Concrete: 2,568.4 kg

(Note: [1]The fraction of an ethanol fermentation plant with an annual throughput of 90,000 tonnes)

5.3 Results and discussion

5.3.1 Energy return on investment

Calculating the energy return on investment is a useful metric for understanding and comparing the sustainability of an energy product in terms of its energy gain. It is desirable that the energy product generates a net gain in energy, which corresponds to an EROI value above one. According to Hall *et al.* [44] for an energy product to be considered sustainable, a minimum EROI of three is necessary. The greater the value of EROI, the more sustainable the source of energy is with a lower depletion of finite fossil energy [224]. Figure 5-2 displays the EROI values for the processing streams of bioethanol production (P1), electricity from biogas production (P2) and bioethanol plus electricity from biogas production (P3) together, respectively, for the base case conditions for each cultivation scenario. The calculated EROI values for petroleum, bioethanol from corn and electricity from biogas are also presented in figure 5-2 for reference. These were calculated using data from Ecoinvent [106]. The EROI values were calculated by dividing the sum of 1 MJ of the total energy produced (as the lower heating value of the energy carriers) and the corresponding energy credit of the co-product by the total cumulative non-renewable fossil energy demand to produce 1 MJ. Figure 5-2 includes horizontal lines displaying EROI values for the minimum values for a net energy gain (EROI=1) and for a sustainable energy product (EROI=3) [44].

Figure 5-3 displays the contribution to the cumulative energy demand (CED) and energy produced for each scenario for the production of 1 MJ of bioethanol (P1), electricity from biogas (P2) and both bioethanol plus electricity from biogas (P3), respectively. The contribution of fertiliser as an energy credit is also displayed.

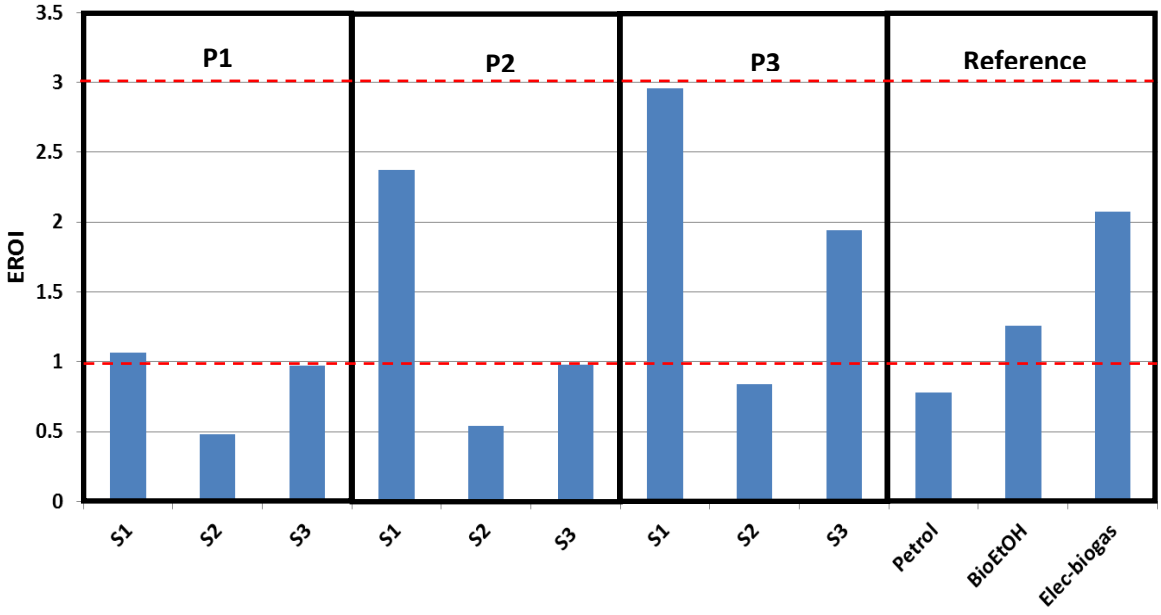


Figure 5-2 The EROI values for the base case conditions of S1, S2 and S3 for bioethanol production, P1, electricity production from biogas, P2, and bioethanol and electricity production from biogas, P3 and reference energy carriers, petrol, bioethanol (BioEtOH) and electricity from biogas (Elec-biogas)

(Note: Reference values use data from Ecoinvent [106], where bioethanol is from corn and the electricity generated from biogas is from the production mix in Switzerland using the allocation factors determined by Ecoinvent)

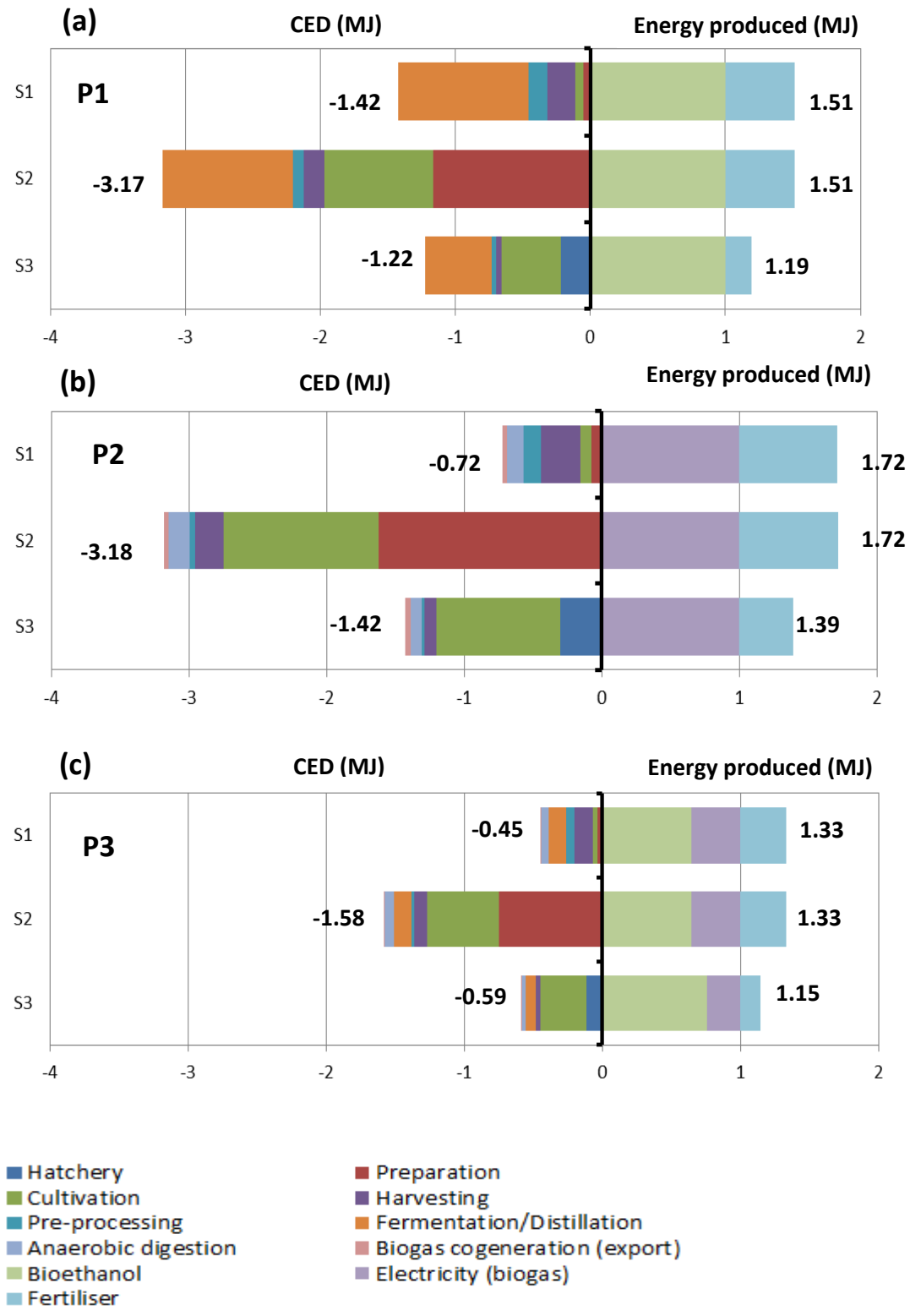


Figure 5-3 Contribution of each unit process to the cumulative energy demand and for the production of 1MJ of (a) bioethanol, P1, (b) electricity from biogas, P2 and (c) bioethanol and electricity from biogas, P3, for each cultivation scenario, S1, S2 and S3

None of the scenarios and process streams reached an EROI value of 3 although bioethanol and electricity from biogas for scenario 1 was only slightly lower (2.95). No process steam allowed for an EROI value above 1 for scenario 2 due to the high CED contributions of the materials for the preparation and cultivation processes (Fig. 3). This method of cultivation was therefore discounted as a possible method of bioenergy generation. The generation of only bioethanol (Fig. 5-3a) produced low values of EROI with only scenario 1 providing an EROI value just over 1, which was lower than bioethanol produced from corn feedstock (1.26) when data from Ecoinvent was used [219]. Such low values suggest the production of bioethanol alone is not worth considering. The high CED of energy generation from the national grid and natural gas led to the low EROI values.

When only electricity from biogas was produced and used for energy generation the EROI value was higher for scenario 1 (2.38) but the value for scenario 3 remained low (0.98) because the value of energy generated was lower and the high CED of material inputs remained. For scenario 1, the production of fertiliser accounted for a large proportion of the energy produced (72%), without this allocation the EROI would be reduced to 1.39. In terms of the cumulative energy demand, the harvesting process accounted for the greatest demand at 40%. For scenario 3, the greatest contributors to the cumulative energy demand were the hatchery and cultivation processes due to the production of the polyamide rope accounting for 56% of the CED.

When both bioethanol and electricity from biogas were produced, the EROI value did increase for scenario 3 but not to the same level of scenario 1. Despite not reaching a value of 3, the production of bioethanol and electricity from biogas for scenario 1 compared favourably to the alternative energy carriers tested with electricity from biogas produced (production mix) having the closest value of 2.07. The greatest contributors for scenario 1 were the harvesting process (30%) mainly due to diesel consumption and the fermentation and distillation process (28%) due to the chemical consumption which accounted for 84% of the fermentation and distillation CED input. As the energy was supplied from the biogas produced, the heat and electricity requirements of both fermentation and distillation and anaerobic digestion were low. Fertiliser accounted for 33% of the energy produced, without

including fertiliser the EROI was 2.22. The EROI value for scenario 3 was 1.94, despite a high energy output the cumulative energy demand of scenario 3 was also high mainly from the hatchery and cultivation processes which contributed 19% and 56% respectively to the CED.

For an alternative comparison, Clarens *et al.* [10] calculated that the EROI value for biodiesel production combined with electricity generated from biogas produced from microalgal biomass ranged from 0.65 to 1.13 under different cultivation conditions. They also tested other processing methods finding direct combustion favourable providing EROI values from 1.53 to 4.09. The values are quite similar to this study although the highest value of direct combustion is higher and suggests that direct combustion of the biomass should also potentially be considered for future research.

5.3.2 Environmental impacts

As the energy analysis favoured the generation of electricity from biogas (P2) and both bioethanol and electricity from biogas (P3) for scenarios 1 and 3, these scenarios and process streams were analysed for their environmental impacts. A wide range of environmental impacts were considered. The results for each category from each scenario were normalised to the highest impact and displayed in Fig. 5-4. The impacts for the generation of petrol, bioethanol from corn and electricity from biogas were also included. The contribution to each impact category for all scenarios is displayed in Fig. 5-5.

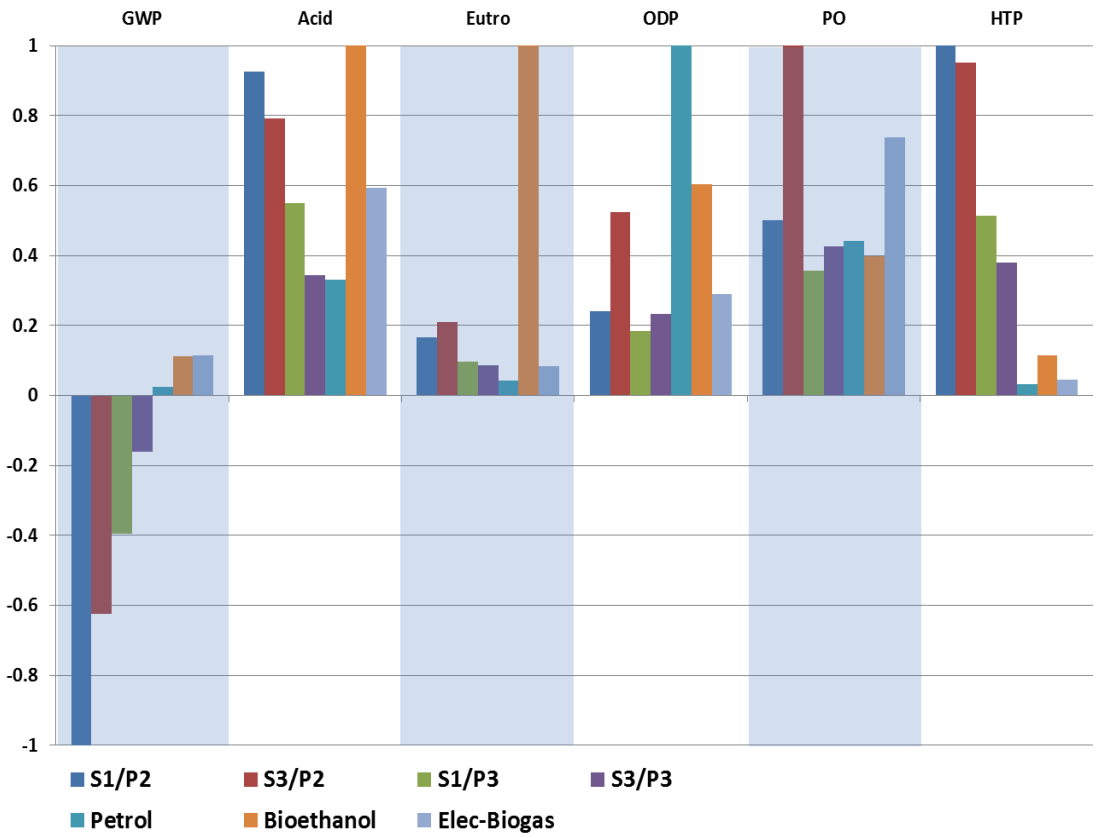


Figure 5-4 Environmental impacts per MJ of energy produced from S1 and S3 for the production of biogas (P2) and bioethanol and biogas (P3) normalised to the greatest impact

(Note: GWP – Global warming potential, Acid – Acidification, Eutro – Eutrophication, ODP – Ozone layer depletion, PO – Photochemical oxidation (summer smog), HTP – Human toxicity potential)

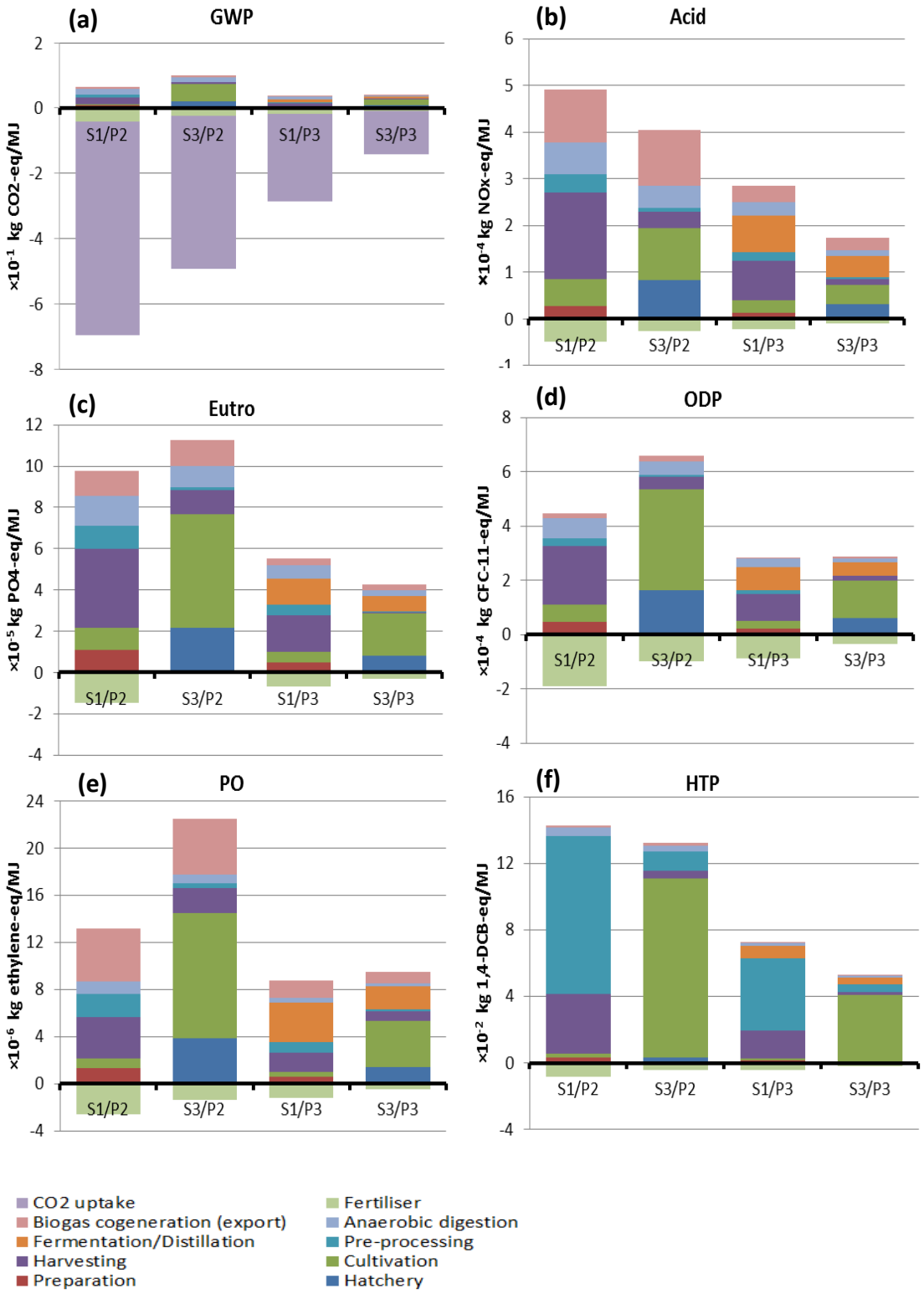


Figure 5-5 The contribution of each unit process to the environmental impacts for S1 and S2 for the production of biogas (P2) and bioethanol plus biogas (P3)

(Note: (a) GWP - Global warming potential, (b) Acid - Acidification, (c) Eutro - Eutrophication, (d) ODP - Ozone layer depletion, (e) PO – Photochemical oxidation (summer smog), (f) HTP - Human toxicity potential)

5.3.2.1 Global warming potential

When considering the global warming potential, both scenarios provided highly promising results. For both process streams the global warming potential was negative as a result of carbon uptake by using a proportion of the biomass for fertiliser. This was in contrast to the alternative fuels tested which produced a net positive GWP value. The highest GWP avoidance was for electricity from biogas for scenario 1, largely as a result of the relatively low energy and high fertiliser yield but also because of low greenhouse gas emissions. The greatest contributors were the diesel consumption for harvesting (23%) and the concrete production of the anaerobic digestion tank (29%). For scenario 3, the high material use, largely a result of rope production for both the hatchery and cultivation processes, led to a higher CO_2-Eq output. The avoided emissions from fertiliser production were also less than for scenario 1. The models did not take into account potential greenhouse gas leakages from the anaerobic digestion facility which can potentially negatively impact the global warming potential particularly when CH_4 is released [225]. Additionally, the emissions associated with fertiliser use were not considered which can also greatly impact the global warming potential particularly due to N_2O emissions [226]. These emissions can be reduced by good farming practice such as ensuring no more fertiliser is applied than is sufficient for crop growth and using efficient methods of application [226]. Another source of green-house gases could be from biomass decomposition during storage which occurs with other biomass sources [227]. Nevertheless as the results suggest, the cultivation and processing of macroalgal biomass could provide a good method of carbon uptake and may be of interest for businesses looking to invest in carbon credits providing an additional source of economic revenue.

5.3.2.2 Acidification

For acidification, each scenario and process stream performed favourably against bioethanol produced from corn but all were worse than petrol. The production of only electricity from biogas performed poorly particularly for scenario 1. This was due to the release of NO_x-Eq from diesel consumption during the harvesting process and biogas combustion for exported electricity production. These processes

accounted for 33% and 23% of emissions respectively. Electricity from biogas generated from scenario 3 was the second least favourable result, due to the emissions from biogas combustion for export electricity generation (30%), polyamide rope production (21%) and the barge production and diesel consumption of harvesting (9%). Methods to reduce diesel use could cut the emissions for scenario 1: a simple method could be finding the optimal cruising speed for distance travelled to fuel usage. For scenario 3, alternative materials for rope production could be sought such as ropes made from natural materials (e.g. polyactic acid and hemp) although these materials tend to have a lower life span.

5.3.2.3 Eutrophication

Each scenario compared well to corn based bioethanol for eutrophication, although the emissions were higher than electricity from biogas (production mix) and petrol. Bioethanol production plus electricity from biogas performed best with similar values for both scenarios due to the higher yields of energy. The material requirements of scenario 3 produced high emissions, particularly from rope production (40%). The high energy yield of bioethanol and electricity from biogas production, however, lessened the impact. For scenario 1 the main source of emission was diesel consumption which similarly to acidification could be reduced by optimising fuel consumption when harvesting. The production of fertiliser has a beneficial impact through avoiding PO_4-Eq emissions from conventional fertiliser production. The eutrophication potential of using the fertiliser, however, was not considered. PO_4 releases could occur as a result of run-off from agricultural land [228]. It is important that leaching is minimised by good agricultural practice such as only applying as much fertiliser as the crop can utilise and the use of efficient application methods [228].

The cultivation of macroalgae in coastal waters offers strong benefits in terms of eutrophication due to the uptake of eutrophication-causing nutrients, mainly nitrogen and phosphorous. Some studies have considered the benefit of macroalgal cultivation for nutrient removal particularly when cultivation areas are located beside fish farms where the nutrient run-off can be reduced [66]. Macroalgae and particularly, *M. pyrifera* and *G. chilensis*, have been found to be effective biofilters [66]. The long-

line cultivation method is more applicable for nutrient removal because lines can be deployed in most coastal areas whereas bottom cultivation is restricted due to depth requirements [60]. Further work needs to quantify this benefit but it should be noted that the potential benefits are great.

5.3.2.4 Ozone layer depletion

Each of the scenarios compared well against petrol and bioethanol from corn for ozone layer depletion. In general, the values were similar to those for electricity from biogas (production mix) with the exception of electricity from biogas for scenario 3 which was higher. As with eutrophication, the main CFC-11-Eq emissions for scenario 3 were a result of the polyamide ropes used, accounting for 64% of emissions. The manufacture of the barge and the diesel consumption also had a high impact. S1 provided the lowest emissions due to the low material inputs, the emissions were mainly a result of diesel consumption. The avoided emissions from fertiliser production also greatly benefited scenario 1.

5.3.2.5 Photochemical oxidation

The values of photochemical oxidation were similar to the alternative fuels tested apart from the production of electricity from biogas for scenario 3 which produced higher emissions. The greatest contribution of kg-ethylene-Eq emissions was from the materials for cultivation, particularly rope production (36%). The combustion of biogas for electricity export also had a high impact (21%); this process also contributed the greatest amount to electricity from biogas for scenario 1. For bioethanol and electricity from biogas, the fermentation/distillation process also contributed greatly due to energy and chemical requirements.

5.3.2.6 Human toxicity potential

Each of the scenarios and process streams performed poorly in comparison to the alternative fuels tested for human toxicity potential. The values were greatly influenced by the production of metals for different processes. In the case of scenario 1, the high value was largely a result of the steel used for the production of the wet biomass attritor accounting for 66% of 1,4-DCB-Eq emissions. For scenario 3, it was the steel chains required for securing the off-shore lines that had the greatest impact,

accounting for 76% of emissions. The production of both bioethanol and biogas from each scenario reduced the emissions as a result of higher yields of energy although the comparison with petrol, bioethanol from corn and electricity from biogas remained poor. Lowering the use of steel would obviously reduce the human toxicity values which could be done by using an alternative material for securing the lines and using the smallest size of attritor for biomass grinding.

5.3.3 Sensitivity analysis

Sensitivity analysis was included to test the impact upon the results of using different specific inputs to the model based on data from alternative sources or using different conditions. The alternative inputs are detailed below and the results are displayed in Table 5-4. The analysis was not conducted for all cultivation scenarios but only for scenarios 1 and 3 for the production of electricity from biogas (P2) and bioethanol plus electricity from biogas (P3). The best case combined all of the input changes.

5.3.3.1 Higher biomass productivity

For the production of *M. pyrifera*, recent studies performed in the South of Chile where selective breeding of biomass gametophytes have been developed have led to very high biomass yields using attachment of the developed gametophytes to long-lines. A value of 80 kg/m (w.w.) was obtained over a 12 month period by Westermeier *et al.* [229]. The corresponding value of 60 t/ha/y (d.w.) was input to the model instead of the base case. For bottom planted *G. chilensis*, a higher productivity value of 145 t/ha/y (w.w.) was tested which was obtained as a result of tri-monthly harvesting in the South of Chile [60].

5.3.3.2 High bioethanol yield from M. pyrifera

Recent research conducted by Bio Architecture Lab using DNA from *Vibrio splendidus* to allow the metabolism of alginate in brown macroalgae achieved high yields (0.281 kg ethanol/kg biomass) of bioethanol from *S. japonica* [115]. Such a yield has not been proven for *M. pyrifera*, but from personal correspondence with Bio Architecture Lab it was understood that such yields are considered possible and therefore this yield was tested.

5.3.3.3 Fewer buoys and longer life span of support ropes

Where biomass was cultivated on long lines off-shore (S2 and S3) the number of buoys were reduced to half of the initial number, 125 buoys/ha. Additionally, the life spans of the support ropes were increased from 5 to 10 years.

5.3.3.4 Fermentation and distillation data

An area of the study where the data used was potentially unreliable was the fermentation/distillation process. As there is no data for ethanol production from macro-algae, the base case used data from Ecoinvent [219] for the production of bioethanol from corn. Alternative data which was used in the LCA study by Alvarado-Morales *et al.* [215] was tested. Their LCA study used data from research where the energy input for the production of ethanol from blue-green microalgae was determined [40].

Table 5-4 EROI and environmental impact values resulting from the sensitivity analysis for each scenario for biogas production (P2) and bioethanol and biogas production (P3).

(Note: GWP-Global Warming Potential, Acid – Acidification, Eutro – Eutrophication, ODP – Ozone Layer Depletion, PO – Photochemical Oxidation (summer smog), HTP – Human Toxicity Potential)

	EROI	GWP kg CO_2 eq	Acid kg NO_x eq	Eutro kg PO_4 eq	ODP kg CFC-11 eq	PO kg ethylene eq	HTP kg 1,4-DCB eq
Base case							
S1 P2	2.38	-0.63	4.41×10^{-4}	8.28×10^{-5}	2.58×10^{-9}	1.06×10^{-5}	1.35×10^{-1}
S3 P2	0.98	-0.39	3.78×10^{-4}	1.05×10^{-4}	5.58×10^{-9}	2.12×10^{-5}	1.28×10^{-1}
S1 P3	2.96	-0.25	2.49×10^{-4}	4.86×10^{-5}	1.97×10^{-9}	7.55×10^{-6}	6.90×10^{-2}
S3 P3	1.94	-0.10	1.64×10^{-4}	4.36×10^{-5}	2.49×10^{-9}	9.02×10^{-6}	5.12×10^{-2}
Higher productivity							
S1 P2	3.10	-0.64	3.52×10^{-4}	6.19×10^{-5}	1.52×10^{-9}	8.56×10^{-6}	9.62×10^{-2}
S3 P2	2.97	-0.42	2.05×10^{-4}	3.86×10^{-5}	1.32×10^{-9}	8.92×10^{-6}	3.46×10^{-2}
S1 P3	3.58	-0.26	2.21×10^{-4}	3.89×10^{-5}	1.48×10^{-9}	6.60×10^{-6}	5.13×10^{-2}
S3 P3	4.77	-0.12	1.02×10^{-4}	1.96×10^{-5}	9.22×10^{-10}	4.60×10^{-6}	1.71×10^{-2}
High bioethanol yield (*M.pyrifera*)							
S3 P3	3.19	-0.23	7.78×10^{-5}	2.22×10^{-5}	1.57×10^{-9}	4.61×10^{-6}	2.72×10^{-2}
Fewer buoys and longer life span of rope							
S3 P2	1.29	-0.41	3.56×10^{-4}	8.89×10^{-5}	4.14×10^{-9}	1.80×10^{-5}	1.27×10^{-1}
S3 P3	2.48	-0.11	1.55×10^{-4}	3.77×10^{-5}	1.95×10^{-9}	7.84×10^{-6}	5.08×10^{-2}
Alternative fermentation/distillation data							
S1 P3	3.74	-0.27	2.15×10^{-4}	4.25×10^{-5}	1.30×10^{-9}	5.27×10^{-6}	6.98×10^{-2}
S3 P3	1.98	-0.12	1.43×10^{-4}	4.31×10^{-5}	2.30×10^{-9}	8.38×10^{-6}	5.48×10^{-2}
Best case							
S1 P2	3.10	-0.64	3.52×10^{-4}	6.19×10^{-5}	1.52×10^{-9}	8.56×10^{-6}	9.62×10^{-2}
S3 P2	3.73	-0.42	1.99×10^{-5}	3.43×10^{-5}	9.30×10^{-10}	8.06×10^{-6}	3.43×10^{-2}
S1 P3	4.85	-0.28	1.71×10^{-4}	3.22×10^{-5}	7.75×10^{-10}	4.25×10^{-6}	5.09×10^{-2}
S3 P3	10.26	-0.04	4.35×10^{-5}	8.38×10^{-6}	3.94×10^{-10}	1.93×10^{-6}	8.95×10^{-3}

5.3.3.5 Discussion of sensitivity analysis

The higher values of productivity increased the EROI value for each scenario and process stream but the greatest increase was for *M. pyrifera* production using the selective gametophyte method [229]. The EROI value for bioethanol and electricity from biogas increased by 246% to a value of 4.77, a higher value than that of scenario 1. This level of productivity has been proven in another study [230] and should be possible on a large scale. Such an EROI value suggests this cultivation method and process stream can provide a sustainable source of bioenergy. The higher yield of biomass and energy production led to lower environmental impacts, particularly ozone layer depletion and human toxicity potential.

Increasing the bioethanol yield of scenario 3 to the value that has been reported for *L. japonica* (28.1 g/g) by Wargacki *et al.* [115] increased the EROI to 3.19 and improved each of the environmental impacts over the base case. The value of acidification potential reduced by 52% and the value of eutrophication by 49%. The improvement meant the scenario compared well in terms of both the EROI and environmental impacts to scenario 1 for base case conditions.

Reducing the number of buoys used and assuming a longer life span of rope had a relatively small impact upon the EROI and did not produce values greater than scenario 1. The environmental impacts for bioethanol and electricity from biogas production were however preferable, apart from photochemical oxidation. As *M. pyrifera* floats it is likely that in practice less buoys could be used.

When the alternative data for the fermentation and distillation process from Alvarado-Morales *et al.* [215] was used there was a 26% increase to the EROI for scenario 1 but little impact upon the EROI for scenario 3. The electricity and heat consumption values for the alternative data were higher than those used for the base case but chemical consumption was not included. The higher energy values made little difference to the EROI and environmental impacts because biogas was used as the energy source but not including the chemicals greatly improved the EROI and environmental impacts for the processing of biomass from scenario 1.

When the best case was modelled, bioethanol plus electricity from biogas production from scenario 3 clearly performed most favourably in terms of the EROI (10.26) and

for all of the environmental impacts. Combining the potentially high biomass yields using selective breeding and assuming a yield of 28.1 g ethanol per g of biomass produced a highly sustainable method of bioenergy generation. These assumptions however have only been proven on a small scale and the high bioethanol yield has not yet been recorded for *M. pyrifera*. Nevertheless, if the cultivation method can be scaled up and if the method for high ethanol conversion efficiency can be applied to *M. pyrifera*, a similarly high value of EROI could be possible.

5.3.4 General Discussion

This study investigated the sustainability of local cultivation of seaweed in Chile and the subsequent production of bioenergy. When considering the EROI values, the results showed that the use of long-line cultivated *G. chilensis* was an unsustainable method of cultivation regardless of the subsequent processing stream. This scenario was therefore disregarded as a potential cultivation method. In terms of the processing streams, production of only bioethanol using any of the cultivation scenarios was very poor and the least sustainable processing method. The EROI results also demonstrated that electricity from biogas would only be sustainable when bottom cultivated *G. chilensis* was used. When both bioethanol and electricity from biogas were considered the EROI values were higher with a marked increase for long-line cultivated *M. pyriferia* which produced a net energy gain. In terms of EROI, the most favourable scenarios and process streams in decreasing order are as follows: S1/P3, S1/P2 and S3/P3.

In terms of environmental impacts the production of electricity from biogas for scenario 1 was poorest in comparison to S1/P3 and S3/P3 mainly due to the low energy production and therefore high relative emissions. Conversely the beneficial negative global warming potential appears to be best because of the high CO_2 uptake relative to the low energy yield. The environmental impacts of S1/P3 and S3/P3 are similar, despite a lower energy yield for S1/P3, the environmental impacts for cultivation and processing were also lower.

When considering base conditions it seems that S1/P3 was the best option. However the limitations of the study must be considered. The sustainability of this scenario was greatly benefited by the production of fertiliser as the digestate. If the digestate

was found to be less effective than assumed in this study or indeed unusable, the sustainability of bioenergy from this scenario would be reduced. Additionally, the cultivation area suitable for producing bottom cultivated *G. chilensis* is likely to be more restricted than the area which could be allocated for long-line cultivation of *M. pyrifera* due to depth requirements and the need for good accessibility. The high labour costs of *G. chilensis* planting and harvesting were not included but would have a high impact upon the economics of the cultivation method.

For these reasons, the long-line cultivation of *M. pyrifera* and processing to bioethanol and electricity from biogas is likely to be preferable as cultivation can be conducted on a large scale on much of the coastline with relatively low labour inputs. When advanced techniques for cultivation and processing of the *M. pyrifera* were considered as part of the sensitivity analysis the sustainability of the scenario with processing to bioethanol and electricity from biogas was by far the most promising.

The two major environmental benefits of development are the uptake of CO_2 and the uptake of nutrients from the coastal waters. The impact of the CO_2 uptake may be limited unless large scale development takes place. The possibility of carbon sequestration however is interesting for businesses that are looking to offset carbon emissions and may be willing to invest in such developments.

For nutrient uptake the most effective method is cultivation near fish farms [66]. The effluents from fish farms can be a cause of local eutrophication leading to anoxic conditions and algal blooms [231] ultimately producing a highly negative impact against the fish farm and the coastline as a whole. As macroalgae have been proven to utilise the nutrients that cause eutrophication to occur [231], the impact could be greatly lessened improving the coastline for all users but particularly fisherman and fish farmers. It should also be noted than an increase in marine biomass may lead to greater shoreline biodiversity [19] as refuges are created for local fauna.

In terms of the socio-economic benefits, the cultivation of the macroalgae can potentially be conducted by local fisherman or fish farmers providing the dual benefit of an extra source of income by selling the bioenergy and fertiliser produced as well as treating the nutrient run-off from the fish farming activities [231]. The

cultivation phase of the system is not particularly intensive and it can therefore be conducted alongside fish farming or artisanal fishing [194]. Equipment such as boats and sheds could be shared between the activities. When a high volume of labour is required (planting/deployment, harvesting etc.,) local fishermen could also be employed providing an extra income and a form of wealth distribution. The skills related to cultivation could be developed easily and taught to allow expansion to other local communities.

This study focussed upon the sustainability of generating bioenergy from macroalgae. The processing of the biomass to products such as agar or alginate was outside the scope. Economic analysis was not conducted as part of this study and the value of the potential bioenergy produced from the biomass should be compared to alternative end products. Combining the production of other products alongside the recovery of energy could potentially improve the sustainability and economic viability of the energy produced [42] and would merit further research.

5.4 Conclusions

Macroalgae has not received as much attention as microalgae for conversion to bioenergy and life cycle assessment studies are very limited. This study considered the cultivation and processing of species common to Chile. Using local data and knowledge a life cycle assessment was conducted to determine the sustainability of several cultivation and processing methods. It was found that the production of bioenergy from macroalgae cultivated in Chile can in most cases yield a positive net energy balance and environmental impacts that are lower than fossil fuels and conventional bioenergy sources. Using current techniques (the base case), bioethanol and electricity from biogas produced from bottom cultivated *G. chilensis* was calculated as being the most sustainable cultivation and processing method. For this scenario an energy return on investment of 2.95 was determined alongside relatively minimal environmental impacts. In the longer term, however, with improved cultivation and processing techniques, the production of bioethanol and electricity from biogas produced from long-line cultivated *M. pyrifera* was determined to provide a considerably more sustainable method as a result of high productivity and ethanol recovery. In the best case an energy return on investment of 10.26 was

calculated. The flexibility of development location and size would also favour long-line cultivation of *M. pyrifera.* These results suggest that continued research in this area would be valuable to maximise biomass productivity and energy recovery.

6 Discussion

6.1 Review of the current state of the knowledge

6.1.1 Microalgae

As far as the author is aware, there are currently no large scale commercial plants producing bioenergy from algal biomass anywhere in the world. The current processing costs do not allow the concept to be competitive with more conventional forms of energy. In terms of the process sustainability, research is still very much in its infancy. There have been a number of life cycle assessment (LCA) studies that have been conducted, many of which suggest that the current common process streams have a negative energy balance or energy ratio [32, 35, 37] and some that suggest under certain circumstances the energy balance or energy ratio can be positive [10, 45].

Most of the studies assume that a relatively high volume of biofuel or energy can be recovered from algal biomass. However, the high processing costs limit the sustainability in terms of the energy balance/energy ratio. The greatest energy consuming processes tend to be fertiliser production, biomass harvesting, drying and the energy consumed within the conversion processes [35, 45].

To grow at its maximum potential, algae must have an abundance of nutrients available. Successful cultivation therefore requires high nutrient addition leading to high fertiliser requirements. Fertiliser production is a notoriously highly energy intensive process [232] and greatly affects the energy balance of the overall system. Lardon *et al.* [35] found that fertiliser production contributed 14.2% to the energy consumption of the bioenergy production system. Clarens *et al.* [27] calculated that a higher percentage of energy use was a result of fertiliser use, almost 50% of the total energy usage. In chapter 4 it was assumed that despite wastewater being used as the main source of nutrients, 1,812 kg/day of superphosphate would be required to avoid phosphorous limiting conditions. It was calculated that this input contributed to up to 30.9% of the energy consumption. When the avoidance of the superphosphate was tested, the energy balance was calculated to increase by up to 42.1% .

Not only does the use of fertiliser greatly impact the energy balance/ratio but it also greatly affects the environmental impacts due to the chemical use of the production process. The CO_2-Eq emissions of fertiliser production is very high, Ecoinvent [106] report that the value of CO_2-Eq emissions of single superphosphate are 2.62 kg/kg superphosphate using the CML 2001 method and for ammonium nitrate the value is 8.55 kg/kg N. In chapter 4 it was calculated that the superphosphate use contributed up to 25.7% of the GWP emissions. Fertilisers not only contribute to the global warming potential but also to most environmental impacts particularly eutrophication and acidification. In chapter 4 it was calculated that the superphosphate contributed up to 32.3% of the acidification potential.

The harvesting method is generally a process which is considered one of the greatest obstacles to successful sustainable bioenergy production from algal biomass. Due to the microscopic size of many species of algal biomass, harvesting can be problematic and a large consumer of energy. Filtration is possible for some species although these species are generally limited to those which a cell size greater than 70 µm [83] which rules out many species. Ultrafiltration is a possible method although issues with material and energy intensity as well as fouling need to be addressed (Zhang *et al.* 2010). A more comprehensive method of harvesting is through centrifugation which can recover high proportions of biomass [82] although this process requires a high consumption of electricity of around 1 kWh/m^3 of water. It was calculated by Sander and Murthy [45] that the harvesting process accounted for 92.7% of total energy demand compared to 86.6% for use of a belt filter press. In contrast Collet *et al.* [33] calculated that the centrifugation step only accounted for 6.6% of the energy demand, a much lower value than the pumping (23.9%) and paddlewheels (31.2%). The high energy demand of centrifugation also results in high environmental impacts through the electricity generation, although this depends upon the method of electricity generation. The use of biogas is likely to result in much lower environmental impacts than conventional electricity production (coal, natural gas, liquid fuel etc.).

Processing of the biomass can be one of the greatest limitations of any system to recover energy from algal biomass. The most effective removal of lipids occurs when

the biomass is dry and therefore drying is advantageous although the energy requirement tends to be high [35]. Lardon *et al.* [35] found that under their base case conditions the biomass drying accounted for 84.9% of the total energy consumption. Again, similarly with the electricity generation the emissions associated with the heat generation depends upon the method employed. If co-generation of biogas is used, the emissions may be very small as well as the extra energy requirement as the heat is a co-product alongside electricity generation. The actual conversion of the biomass to energy generally requires little energy and materials although this depends greatly upon the method employed. Lipid extraction and trans-esterification requires mainly heat and electricity for both processes, hexane for oil extraction and methanol for trans-esterification [35]. With regards to the preceding processes the consumption of electricity and heat is relatively small. Lardon *et al.* [35] calculated that the electricity and heat for both processes was 8.9% of the total energy demand for the base case. Compared the cultivation process, Stephenson *et al.* [32] also found that the energy consumption of the lipid extraction and esterification was very low. Much of the chemical materials can also be recycled, Stephenson *et al.* [32] assumed a hexane recovery rate of 50%, as did Gao *et al.* [233].

The production of bioethanol requires more heating than biodiesel production to allow fermentation to occur as well as purifying the ethanol through distillation. A benefit however is that the biomass does not require drying as the process is wet. In chapter 4 when the production of bioethanol from the harvested algal biomass was tested, it was calculated that the fermentation and distillation process accounted for 18.2% of the energy demand, the energy requirement (heat and electricity) accounted for 42.6% of this demand. When bioethanol production followed the extraction of lipids the fermentation and distillation process accounted for 12.9% of the total energy demand. The energy demand not a result of the electricity and heat consumption was mainly a result of the chemical consumption. The chemical consumption also results in high environmental impacts.

Anaerobic digestion of algal biomass is a comparatively low impact process in comparison to biodiesel and bioethanol production. The biomass does not require drying and no chemical inputs are necessary. The anaerobic digestion of the biomass

is therefore not a limitation, indeed it is a process that can be used to produce energy either directly from the biomass or from the residual waste following prior processing.

Limitations to the production of bioenergy from algal biomass that are not considered as technical as the processing limitations are:

- The requirement of a large area
- The requirement for freshwater
- The demand for the energy products

One of the major benefits of recovering energy from algal biomass is that arable land is not a requirement for growth. However when raceway ponds are used the areal requirement is relatively high [37]. Ideally the use of arable land would be avoided in any development to cultivation algal biomass. If the scenario suggested in chapter 4 is used (where the cultivation of algal biomass is used for wastewater treatment), the use of arable land may be unavoidable or indirect land use change may affect arable land.

In cases where freshwater is used as opposed to wastewater, the water use is very high due to the nature of algal cultivation. To produce 317 GJ of algal biomass, Clarens *et al.* [27] calculated that 120,000 m^3 of water would be necessary. The other bioenergy crops tested required a far lower consumption of water (8,200, 10,000 and 5,700 m^3 of water for corn, canola and switchgrass respectively). Many parts of the world are currently facing water scarcity [234] and therefore are perhaps not appropriate locations for the cultivation of algal biomass as freshwater is necessary. Indeed, areas which favour algal cultivation tend to have warm, dry climates and therefore are likely to have particularly water scarcity issues. The obvious solution as suggested in chapter 4 is to use wastewater instead of freshwater. Obviously appropriate wastewater is relatively limited and therefore widespread uptake of the concept is unlikely.

For much of the ideas put forward in this thesis requires the proposed energy products to be in high demand. If there is no demand for the products then the system will not be economically valuable. As clean sources of energy are, in general, greatly sought after, in most cases this is unlikely to be a problem although the energy has to

be able to be produced for a similar cost to conventional energy carriers. Bioethanol is probably the energy carrier that is likely to be least sought after because it cannot be used as a direct substitute for conventional fossil fuels like biodiesel can [235]. In the majority of engines it can be used directly at a concentration of 5% although in specialised energy it can be used at a concentration up to 85%. The use of these engines, however, is not widespread [236]. Biodiesel has the benefit that it can be used as a direct substitute for diesel [235] and therefore the value should be similar. Biogas is not generally considered a particularly high value energy product however it can be used in a biogas co-generation engine to produce both electricity and heat, both of which have a high value [219]. A problem that may jeopardise the conversion of algal biomass to energy is that the value of the biomass to be used for different purposes (pharmaceuticals, foodstuff etc.) may be higher than the energy product and therefore would be more worthwhile. This thesis did not consider the economics of energy generation from algal biomass in any detail but this is a particularly important area considering the long term sustainability and must be researched in great depth.

6.1.2 Macroalgae

In general the cultivation of macroalgae has far lower inputs and is less energy intensive than the cultivation of microalgae however productivities also tend to be lower [19]. As mentioned in chapters 2 and 5, the method of macroalgae cultivation depends upon the species. In chapter 4, bottom planting and long-line cultivation were considered. The greatest inputs to the long-line cultivation system were the ropes required for spore inoculation which accounted for the greatest energy consumption and environmental impacts. In chapter 5 it was calculated that for the production of bioethanol and biogas from long-line cultivated *Macrocystis pyrifera*, the rope production accounted for 50.5% of the total cumulative energy demand. Similarly, large percentages were also calculated for each of the environmental impacts that were determined due to the rope input. The steel chains used to retain the position of the long lines also contributed greatly to the environmental impacts due to the intensity of steel production. Polyamide was the material assumed to be used for the cultivation line and structural lines. It is possible that different materials could be used to manufacture the culture and structural lines to reduce the impact. Natural materials such as hemp, cotton or manila could potentially be used although

their life span is likely to be considerably less than that of ropes made from plastics. Similarly, the steel chains anchoring the array could be replaced by strong plastic ropes although again, the life span may not be as long. Very high EROI values for the production of bioenergy from long-line cultivated *M. pyrifera* were calculated when the highest recorded conversion rate of brown algae to ethanol was assumed. The method employed to allow this conversion was technically complex in comparison to conventional conversion methods and it is likely that the energy and material use would be higher as a result. Additionally, the method has been proven only for one species of macroalgae (*Saccharnia japonica*) and may not be suitable for other species. If this method cannot be used then the EROI and environmental impacts of bioethanol generation are likely to be very low and high, respectively. Conversion rates for macroalgae to bioethanol using conventional techniques are generally low due to the difficulty of hydrolysis of the polysaccharides [114]. If more advanced techniques are unable to be used, the sustainability of the system would be greatly reduced.

The technique of bottom planting is entirely different from long-line cultivation and the limitations are accordingly different. The species that was investigated in chapter 5 for bottom cultivation was *Gracilaria chilensis* which is a species commonly cultivated using this method in Chile. Using base case inputs, in chapter 5, the generation of bioethanol and biogas from bottom cultivated *G. chilensis* appeared fairly sustainable although many of the limitations were not considered quantitatively. The depth of culture greatly affects the productivity of the biomass: the area that can used for cultivation is therefore restricted to those areas with a depth between 0.75 to 2.5 m [60]. Apart from the restriction of areas, the preparation and planting process is highly labour intensive which although increases local employment, it also increases the energy consumption and environmental impacts of the system. Given the nature of the preparation and planting, it is a difficult process to mechanise, although some techniques have been developed to plant thalli using an automatic injection method. Similarly, the harvesting of bottom planted biomass is intensive requiring divers to handpick the thalli from the sea floor which is considered the best method for high subsequent productivity [60]. It was understood from local *G. chilensis* farmers in Chile that it can take a diver around 3 days to

harvest one hectare (Personal communication with seaweed farmers, March 2013). There are aquatic vehicles that are capable of harvesting bottom planted biomass (RS-Planering Ltd, [156]) although obviously the energy requirements and environmental impacts of producing and operating this sort of vehicle are likely to be high. Like many seaweeds, *Gracilaria* does not provide a particularly good substrate for bioethanol production and only low yields have so far been obtained [117]. It appears that methane production can be high from *G. chilensis* [126] and should be incorporated into any bioenergy process stream.

Another limitation that applies to both methods of macroalgae production is the presence of epiphytism, which is the impact of parasites upon the biomass. This has been observed in many cultures [237, 238] and was a problem that was highlighted by the company Bio Architecture Lab in Chile through private communication. Figure 6-1 shows a photograph of the effect of epiphytism on a piece of *Macrocystis pyrifera* thalli. Epiphytism can severely reduce biomass productivity or indeed completely destroy whole crops which would obviously jeopardise the sustainability of any bioenergy productions system using macroalgal biomass. Similarly to microalgae, if the value of the macroalgal biomass is greater than that of the produced energy carrier there is little worth in recovering energy. This was in fact the case for the Bio Architecture Lab project in the south of Chile where the production of bioethanol was proposed, the project is no longer continuing [239].

Figure 6-1 Epiphytism of a piece of *M. pyrifera* thalli observed on a beach in Chiloe Island, South Chile

6.2 Investigation of the growth of indigenous species in wastewater using open systems

In chapter 3 the cultivation of locally obtained freshwater algae in agricultural effluent was tested yielding very positive results. The results from this chapter showed that in each dilution of sterilised swine effluent the algae was able to develop whilst reducing the nutrient concentrations within the effluent. Reductions in NH_4^+ were calculated to be at least 97% and 54% for PO_4^{3-}. Despite the positive results it was also found that there was a certain degree of nutrient removal in the blank containers suggesting some other microbial interaction. Furthermore the results are not particularly indicative of a large scale scenario due to the conditions used within the study. Firstly, the cultivation was conducted inside the laboratory and therefore there was little likelihood of contamination from competing organisms which was shown to occur in the outdoor cultures that were set up. Additionally, artificial lighting was used to substitute for natural light. It would be unlikely in an outdoor environment that such a consistent source of light would be available although this could be the case in some parts of world where sunlight is more consistent than Scotland. Although the productivity was calculated to be relatively low the large cell

size of the algae favours easy harvesting as was demonstrated by removal using tweezers, a low impact method on a larger scale would be easily implemented.

The work conducted in chapter 3 shows that there is strong potential for the cultivation of local freshwater algae in agricultural effluent which supplied nutrients and allowed the uptake of the nutrients producing cleaner water. Harvesting was also shown to be very easy for this particular species. If the concept was used on a larger scale however, nutrient addition may be necessary to stimulate a greater production of biomass and contamination issues may arise.

6.3 Investigation of the conversion of algal biomass using biological and thermal processes

In chapter 3, as well as testing the cultivation potential of locally obtained algal biomass, biomass was also tested for its energy recovery in the form of bioethanol as well as pyrolysis products. The hydrolysis and fermentation section compared several biomass feedstocks, of which the freshwater algal biomass performed most favourable in terms of the glucose recovered and the ethanol produced. The filamentous characteristics suggest that the biomass contains a high proportion of polysaccharides. The lack of lignin in algal biomass means the hydrolysis of these polysaccharides should be converted to glucose, the results obtained in chapter 3 support this. In contrast, the glucose and ethanol recovery from the brown macroalgae was very low due to the difficult hydrolysis of much of the polysaccharides contained within brown algae (mannitol, laminarin etc.) which was mentioned in 6.1.2. The results suggest that bioethanol recovery is a good conversion method for this particular species of freshwater algae but conversion of macroalgae faces a greater challenge.

The pyrolysis of the biomass was also tested for the production of biochar and other pyrolysis products (syngas and bio-oils). The algal biomass was compared to a synthetically produced municipal solid waste. In terms of energy recoverability, the municipal solid waste provided the best feedstock as a greater amount of bio-oil and energy rich gas was produced. The algal biomass provided a higher recovery of biochar meaning greater potential as a soil amender and for carbon sequestration

although for the method used the biomass would first require drying which given the high moisture content of the algal biomass would likely jeopardise the viability.

6.4 Investigation of the sustainability of incorporating algal cultivation and conversion to energy into the wastewater treatment process as a method of nutrient removal

It has been suggested in many studies that the use of nutrients in wastewater streams for algal cultivation will greatly improve the sustainability of the system for bioenergy generation [27, 204]. Chapter 4 investigated the cultivation and processing of algal biomass in secondary treated wastewater for nutrient removal in comparison to a more conventional technique. The LCA that was conducted was based on many assumptions and under the base case it was calculated that the cultivation of algal biomass would be preferable to conventional nutrient removal in terms of the energy balance and two of the three environmental impacts (global warming potential and eutrophication). Despite such positive results there were assumptions used in the model that may not be the case on a realistic system. The productivity of the algal biomass was based on research conducted in Israel, however obviously in locations with a climate less suitable to growth the productivity and therefore nutrient removal capabilities would be greatly reduced which would have an obvious impact upon the sustainability. Additionally, an average productivity value was used which would not be the case as research has shown biomass productivity to vary greatly between different times of the year [47]. In winter, the productivity would be likely to be very low meaning nutrient removal would be ineffective and a back-up method of nutrient removal would therefore be necessary for when the productivity is too low. This would result in extra financial investment as well as the associated increase in energy requirement and environmental impacts. As well as seasonal low productivities, another limitation would be the varying nutrient concentration levels in the wastewater which would also lead to varying productivities and potentially the necessity to constantly alter the nutrient supplementation to avoid nutrient limitation. An important limitation that has been mentioned previously is the competition from other organisms which can freely develop in the open ponds and have a greatly negative impact upon the operation and therefore the overall sustainability. This is a

difficult issue to overcome that could potentially only be mitigated through the use of pesticides although these may not be completely effective and would also entail a high environmental impact or through covering the ponds which would require an extremely high material input. Further work is necessary to understand the impact of competing organisms upon algal biomass in open ponds.

Chapter 4 looked specifically at a municipal wastewater stream in a particular location. There are however many other wastewater types that may be applicable for the use of algal cultivation for nutrient removal and biomass production in parallel. The work in chapter 3 showed that swine effluent can make an excellent source of nutrients and the same is likely for other agricultural effluents with high nutrient loading. The issue with many of these effluents is likely to be that the nutrient concentrations are too high and require dilution which means a source of freshwater would be necessary which may impact the local environment. The benefit of nutrient removal in these cases may also not be as high as that for municipal solid waste and the value therefore of the algal cultivation providing this benefit would be lower. There are many other wastewater streams that may provide opportunities for algal cultivation, such as carpet wastewater [240, 241], brewery/distillery wastewaters [160, 242-244] and refinery wastewaters [245] among others. The problem with these more industrial effluent streams is that they contain many strong contaminants that may adversely affect the growth of algal biomass and the nutrient concentrations are likely to be much lower than those of agricultural effluents. In this respect, the wastewater is likely to need a greater degree of treatment prior to the use of algal ponds however it is likely that this would be required regardless.

6.5 Investigation of the sustainability of macroalgal cultivation and processing to bioenergy

As mentioned at the beginning of the thesis, most of the research looking at bioenergy recovery from algal biomass has focussed upon freshwater microalgae with macroalgae receiving little attention in comparison. Freshwater microalgae has received the majority of attention due to its productivity and capacity for producing biodiesel. Macroalgae, however, also has many benefits. Chapter 4 investigated the recovery of bioenergy from macroalgal biomass using Chile as a case study. The

benefits of macroalgae cultivation are the low inputs required for cultivation (in some circumstances), the very low land requirement, little, if any fertiliser consumption, nutrient uptake potential and employment potential in rural areas. Macroalgae has been shown as being suitable for large scale cultivation in many parts of the world [19, 114, 229]. Additionally, many species show exceptional growth rates under favourable conditions and can therefore be considered good sources of biomass for energy generation for relatively low inputs. The inputs however depend greatly upon the method of cultivation. The work in this thesis looked at two methods: bottom cultivation and long-line cultivation. Long-line cultivation was found to provide the greatest productivities. However the relatively low input of bottom cultivation meant that this appeared to be the most sustainable option. For reasons mentioned in 6.1 however, it is more likely that long-line cultivation will provide the best method due to greater applicability. Additionally, the potential improvements to the long-line cultivation are more promising than those of bottom cultivation as demonstrated in chapter 5.

The potential energy products from macroalgae are less than those from microalgae due to the characteristics of the biomass, generally containing more polysaccharides than lipids. The favoured energy carriers are bioethanol and biogas. The problem with bioethanol production, as mentioned in 6.1, is the difficulty to hydrolyse many of the polysaccharides. If cost-effective methods can be found to recover high yields from different types of macroalgae that have low environmental impacts then there is definite potential for this concept to be developed further. The generation of biogas from macroalgal biomass appears to offer a good alternative as a much simpler process, although the value of biogas is low unless there is a potential use for the heat generated in parallel to the generation of electricity. Providing the value of the energy produced is greater than that of the alternative potential products from cultivated macroalgal biomass, it is likely that many sustainable systems can be developed around the world to cultivate and convert macroalgal biomass to bioenergy. If the market dictates that the biomass should be used for non-energy purposes then the recovery of energy from the residual biomass should be investigated.

6.6 Comparison of the sustainability of the different cultivation and conversion strategies and identify what further research is required

This thesis aimed to investigate the sustainability of several different methods to recover energy from algal biomass. The main focus was the cultivation of freshwater algae in a wastewater stream and subsequent recovery of biodiesel, bioethanol and biogas. The cultivation and conversion of macroalgae to bioethanol and biogas was also considered. The scenarios considered were entirely different however the LCA method used was similar for both and also some of the same impact categories were used. A comparison of the values for the EROI and several of the common impact categories is shown in table 6-1. Only the base case values were used for simplicity. For the values taken from the study investigating the cultivation of freshwater algae, the EROI was calculated using the same method as that in chapter 5 as the energy balance and not the EROI was calculated in chapter 4. Furthermore, the energy consumption and environmental impacts of the wastewater treatment processes were not included when the values were calculated to allow a fair comparison to be made. The details of each scenario number are displayed under the table.

Table 6-1 The values of EROI and environmental impacts for each scenario considering the cultivation and processing of both freshwater algae and macroalgae

(Note: 1-4: Freshwater algae cultivated in secondary treated wastewater, 5 & 6: *G. chilensis*, bottom-cultivated in Chile, 7 & 8: *M. pyrifera*, long-line cultivated in Chile)

No.	Energy product	EROI	GWP (kg CO_2-Eq)	Acid (kg SO_2-Eq)	Eutro (kg PO_4-Eq)
1	Biodiesel and biogas	1.78	-0.11	0.00031	0.000082
2	Bioethanol and biogas	0.70	-0.20	0.00092	0.00023
3	Biogas only	0.042	-11.12	0.016	0.0037
4	Biodiesel, bioethanol and biogas	1.77	-0.028	0.00032	0.000087
5	Bioethanol and biogas	2.96	-0.25	0.00026	0.000049
6	Biogas only	2.38	-0.63	0.00044	0.000083
7	Bioethanol and biogas	1.94	-0.10	0.00016	0.000044
8	Biogas only	0.98	-0.39	0.00038	0.00010

The results indicate that the recovery of bioenergy from macroalgae is the best method in terms of the sustainability, particularly the bottom cultivation of *G. chilensis*. As noted earlier, however, it is unlikely that this cultivation method could be scaled up to any large development and therefore the long-line cultivation of *Macrocystis pyrifera* is probably a better option. For *M. pyrifera*, only the production of bioethanol and biogas was calculated to have an EROI above one. The EROI for the production of biodiesel and biogas from freshwater algae grown in wastewater was similar at 1.78. The production of bioethanol and biogas and biogas only were calculated to be not worth considering given EROI values below 1.

The environmental impacts were within the same region for each scenario. Bioenergy from macroalgae performed better in terms of the global warming potential (although not compared to only biogas produced from freshwater algae but the low EROI makes this scenario redundant) due to much of the biomass and therefore the carbon being used as fertiliser. The values for acidification were very similar with bioethanol and biogas from *M. pyrifera* performing the best by a small margin. This scenario also provided the best result for eutrophication although bioethanol and biogas from *G. chilensis* was very similar and only slight better than biodiesel and biogas from the freshwater algae.

The work conducted in this thesis suggests that bioenergy recovered from macroalgae is slightly more sustainable than bioenergy recovered from freshwater algae cultivated in wastewater effluent. The results however consider only several types of environmental analysis and are based on many assumptions that still require further research. This work puts forward a positive case for recovering energy from both freshwater algae and macroalgae in specific circumstances where conditions are favourable however much more research is required to overcome problems that will occur when scaling up each of the concepts. This work also did not consider the economics of any of the systems studied in any great depth and this is a key area for sustainable implementation which must be addressed.

7 Conclusions

The purpose of this thesis was to investigate the sustainability of bioenergy production from algal biomass by looking at the practicalities of implementation and the energy balance and environmental impacts resulting from different systems to cultivate biomass and recover energy. The limitations of algal bioenergy were identified at the beginning of the thesis, the processes were largely split into the cultivation of freshwater algae and macroalgae. The limitations of freshwater algal cultivation were identified as:

- Fertiliser consumption
- Freshwater use
- Contamination of selected species
- Energy use for harvesting
- Low energy yields

Given that the techniques to cultivate and recover energy from macroalgal biomass are quite different from freshwater algae the limitations were accordingly different and were identified as:

- Material consumption (long-line cultivation)
- Energy and labour for planting and harvesting (bottom cultivation)
- Low productivity and energy yields

The general limitations affecting both systems were the material and energy inputs and the low energy recovery. Potential solutions were suggested to improve the sustainability for each process system. For freshwater algal cultivation these solutions included the use of wastewater streams to reduce fertiliser consumption and provide a wastewater treatment benefit plus a reduction in freshwater use, the development of locally dominant species to minimise contamination issues, the use of low impact harvesting methods (such as flocculation with chitosan) and the maximum recovery of energy by considering several conversion streams to different energy carriers.

The cultivation techniques for macroalgae are less flexible than those for freshwater algae and are more difficult to improve. A method to provide extra environmental and social benefits is the cultivation of macroalgae alongside fish farms where the uptake of run-off nutrients can increase biomass productivity and remove nutrients that would otherwise cause pollution. Two methods of biomass cultivation were suggested, long-line cultivation which is more widespread but has a high material input and bottom cultivation which can potentially reduce the material inputs although the intensity of cultivation is high. Using the best processing method for macroalgal biomass is also important in terms of the sustainability to maximise the energy recovery as processing of macroalgal biomass can be problematic.

Chapter 3 looked specifically at the practicalities of freshwater algal cultivation and processing by conducting experiments examining these processes. The results were very positive for those conducted within the laboratory. A maximum growth rate of 0.314/day was achieved when a local species of *Spirogyra* sp. was cultivated in agricultural effluent. The growth of the biomass also reduced the nutrient loading of the effluent by up to 98% for NH_4^+ and 90% for PO_4^{3-} although the results showed that there may also have been some microbial interaction due to partial nutrient removal in the blank samples (i.e. in the absence of algae). These results should be replicable on a larger scale although as demonstrated by outdoor cultivation experiments also presented in chapter 3, the influence of competing organisms may well cause problems. One benefit of using local species of algae is that they are the dominant species of the location in which they are found and in many cases (such as chapter 3) they can be harvested easily due to their relatively large cell size compared to many selected species of microalgae.

The algal biomass obtained in chapter 3 provided an excellent substrate for conversion to bioethanol comparing extremely well to the alternative biomass types tested. A maximum bioethanol recovery of 35.8% was recorded which suggests that the biomass is a good feedstock for bioethanol recovery. The hydrolysis and fermentation methods used can be considered fairly conventional and therefore similar yields should be achievable for the species used and other, similar species of freshwater without the requirement for technologically advanced and expensive

techniques. The brown macroalgae tested proved a poor substrate highlighting the difficulty of converting macroalgae to bioethanol. The freshwater algal biomass proved a poor substrate for bioenergy recovery through pyrolysis compared to the alternative biomass tested. Pyrolysis offers a method of carbon sequestration although drying the biomass may reduce the effectiveness.

The experimental part of the thesis showed that there is great potential for cultivation of local species in agricultural effluent however reinforced the limitations such as contamination, nutrient limitation, low productivity and reliance upon consistent sunlight and warm temperatures.

Chapter 4 investigated the potential of incorporating algal cultivation as a method of wastewater treatment in wastewater treatment plants using an LCA approach and case study. The LCA compared upgrading a wastewater treatment in Israel for improved nutrient removal using either a conventional method (A_2O process) or a novel method (algal cultivation). In terms of sustainability, the technique, under specific conditions, appeared to favour the use of algal cultivation ponds as a positive energy balance was recorded. When the biomass was assumed to be converted to biodiesel, bioethanol and biogas an energy balance of 240,958 MJ was calculated for one days treatment compared to -80,487 MJ for the conventional method. For algal cultivation, each process stream also led to an uptake of CO_2 as opposed to a net release through conventional treatment. The algal cultivation was also beneficial in terms of eutrophication (except the scenario where only biogas was produced) where conventional treatment produced net emissions. The conventional treatment was preferable in terms of acidification but generally speaking was outperformed by algal cultivation and conversion to: biodiesel plus biogas and biodiesel, bioethanol plus biogas.

The model however relied upon several key assumptions: the assumption that high growth would be constant year round, ponds would be free of contamination and the effluent discharge would be free from eutrophication. There is a positive case for further research investigating the use of algal cultivation ponds for enhanced wastewater treatment as a result of the high energy recovery potential as well as the improved environmental impact. New research should develop pilot scale systems to

determine the efficacy of a larger scale system and find solutions to the issues raised in this work, mainly the problem of contamination and overcoming ineffectiveness in less productive months.

The work in chapter 5 considered the cultivation and processing of macroalgal biomass to bioenergy in Chile. The study used a life cycle assessment to determine the sustainability of several cultivation techniques and processing methods by considering the energy return on investment and several environmental impacts. The low impact method of bottom cultivation was determined to be the best option returning an EROI of 2.95 and environmental impacts relatively minimal in comparison to more conventional bioenergy sources. However as discussed in chapter 4 it would be a difficult method to develop on a large scale due to areal limitations. The long-line cultivation of brown algae shows more promise in this respect. Under the base case conditions the EROI was just below 2 for the production of bioethanol and biogas from long line cultivated brown algae. Despite high productivities and good conversion to energy the high material input to the system reduced the effectiveness, When potential improvements to the cultivation and processing of long-line grown brown macroalgae were considered the system appeared to show a very sustainable method of energy generation. The use of newly developed culture techniques yielding high productivity resulted in an EROI value of 4.77 and a considerable reduction in environmental impacts. The inclusion of a high bioethanol recovery yield reported from a specific species of brown algae provided an EROI of 3.19 and a reduction in acidification and eutrophication potentials by around 50%. The focus therefore for future research needs to be upon maximising productivity of the biomass and improvingenergy recovery through new techniques, particularly for bioethanol production. To increase the viability of the process, extra value should be sought from the biomass through production of high value products and as a method of carbon off-setting.

The main conclusions of this piece of work are as follows:

- Many limitations to the cultivation and processing of algal biomass to bioenergy are preventing successful commercialisation of the concept. The main limitations are high material inputs, fertiliser requirements, inconsistent yields and low energy yields.
- Freshwater algal biomass and indigenous species have excellent capacity for cultivation in wastewaters with almost complete reduction of some nutrient loading in the water. The conceptmust continue to be scaled up and tested in specific locations using suitable local strains of algae to overcome issues such as contamination.
- Freshwater algal biomass has shown the potential for successful conversion to many types of bioenergy carriers. Conversion of local filamentous algae to bioethanol yielded recovery rates up to 35.8%. The inputs to the processes to convert the biomass should be limited and the processes optimised. The method of biomass to energy conversion should be selected on an individual basis determined by the characteristics of the biomass. Following pilot scale cultivation at a particular site, different process streams should be tested to find the optimal method to recover the maximum amount of energy for the least energetic input.
- Using algal cultivation as a method of nutrient removal in wastewater treatment plants has the potential to improve the sustainability of the wastewater treatment process.. The energy balance of a wastewater treatment plant operation can be made positive by recovery of energy from the biomass. In addition this will improve the environmental impacts of the treatment plant such as providing an uptake of CO_2 as recorded in chapter 4. The high areal requirement may limit the development of this concept however now investment should be made in pilot scale developments to test specific locations, wastewaters and biomass species. Larger scale tests will also allow further limitations to be identified and overcome.
- Macroalgal cultivation has excellent potential for producing a sustainable source of bioenergy providing cultivation is developed in locations that are capable of supporting it. An EROI of 2.95 was recorded in this thesis for recovery of bioethanol and biogas from *Gracilaria chilensis* and a value as high as 10.26 was

recorded for *Macrocystis pyrifera* when new culture and processing techniques were considered. Further investments should be made into laboratory research to maximise energy recovery yields from the biomass either as virgin biomass or as residual biomass following prior use. Without ensuring high energy yields can be obtained from easily cultivated species it's unlikely that a system to cultivate and recover energy from macroalgae would be viable. Such a system could also provide many positive social and economic impacts to the cultivation area which should be fully quantified.

- Both types of algal biomass can contribute greatly to the global energy demand however development is likely to be limited to locations where the cultivation and processing of biomass can be conducted sustainably. Investments should be made to identify the most suitable locations for developing each concept further. For freshwater algae, this should be the development of pilot scale systems using sites with an abundance of wastewater and a suitable climate. For macroalgae, large scale cultivation has been proven in many locations however further investment is necessary at a lab scale to allow easy recovery of high energy yields which can also be implemented on a larger scale.

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Appendix A: Cultivation of algae and conversion to bioenergy

A.1 Nutrient uptake in agricultural effluent

A.1.1 Calibration curves for ammonium and phosphate measurement

To measure the uptake of ammonium and phosphate in each of the containers it was necessary to develop calibration curves to calculate the ammonium and phosphate concentrations relative to the values of absorbance measured by the spectrophotometer. For the ammonium calibration curve, a series of standards were produced using ammonium chloride. Volumetric flasks containing NH_4Cl concentrations of 0.5, 1, 2, 3 and 5 mg/L were produced. The equivalent concentrations of NH_4 were 0.17, 0.33, 0.67, 1.00 and 1.67 mg/L calculated based on the stoichiometry of NH_4Cl. A 5ml sample of each standard was extracted using a pipette and placed in a small 10 ml volumetric flask. The chemical reagents (supplied by Spectroquant) were then added to the flask and the reaction was took place. A sample from each flask was then placed in a 50 mm quartz cell and the absorbance measured in the spectrophotometer (Thermo Scientific, Helios Alpha) at a wavelength of 650 nm. Each reaction was performed in triplicate. Figure 1 displays the absorbance values measured for each ammonium concentration and Figure 2 displays the corresponding calibration curve.

Table A-1 Values of absorbance measured for each ammonium standard (standard deviation in parentheses)

NH_4Cl (mg/L)	NH_4 (mg/L)	Absorbance			Mean
		1	2	3	
0.5	0.17	0.124	0.127	0.118	0.123 (0.00458)
1	0.33	0.239	0.225	0.237	0.234 (0.00757)
2	0.67	0.442	0.434	0.462	0.446 (0.0144)
3	1.00	0.626	0.649	0.678	0.651 (0.0261)
5	1.67	1.052	1.037	1.053	1.047 (0.00896)

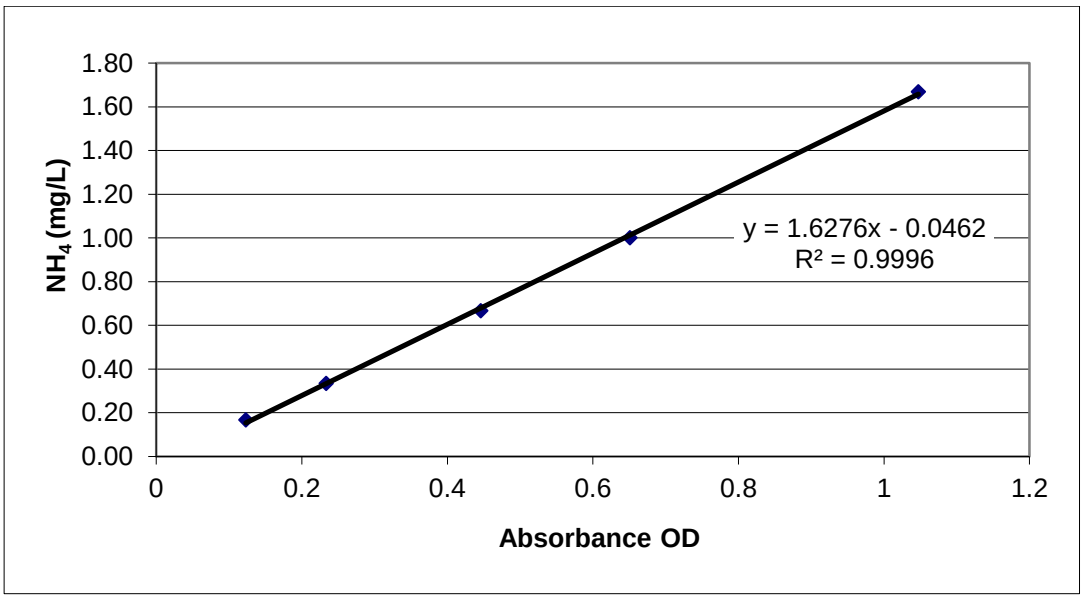


Figure A-1 Calibration curve for ammonium measurements using standards

The calibration curve for the phosphate measurements was conducted using potassium phosphate. Standards of potassium phosphate with concentrations of 1, 2, 3 and 5 mg/L were produced which corresponded to phosphate concentrations of 0.69, 1.38, 2.07 and 3.45 mg/L. The standards were reacted using the same method as used for the ammonium but with the phosphate test kit. Again, triplicate samples were used. Figure A-2 displays the absorbance values for each standard and Figure 4 displays the calibration curve.

Table A-2 Values of absorbance measured for each phosphate standard (standard deviation in parentheses)

KH_2PO_4 (mg/L)	PO_4 (mg/L)	Absorbance			Mean
		1	2	3	
1	0.69	0.071	0.070	0.067	0.0693 (0.00208)
2	1.38	0.134	0.135	0.135	0.135 (0.000577)
3	2.07	0.209	0.211	0.210	0.210 (0.001)
5	3.45	0.327	0.326	0.324	0.326 (0.00153)

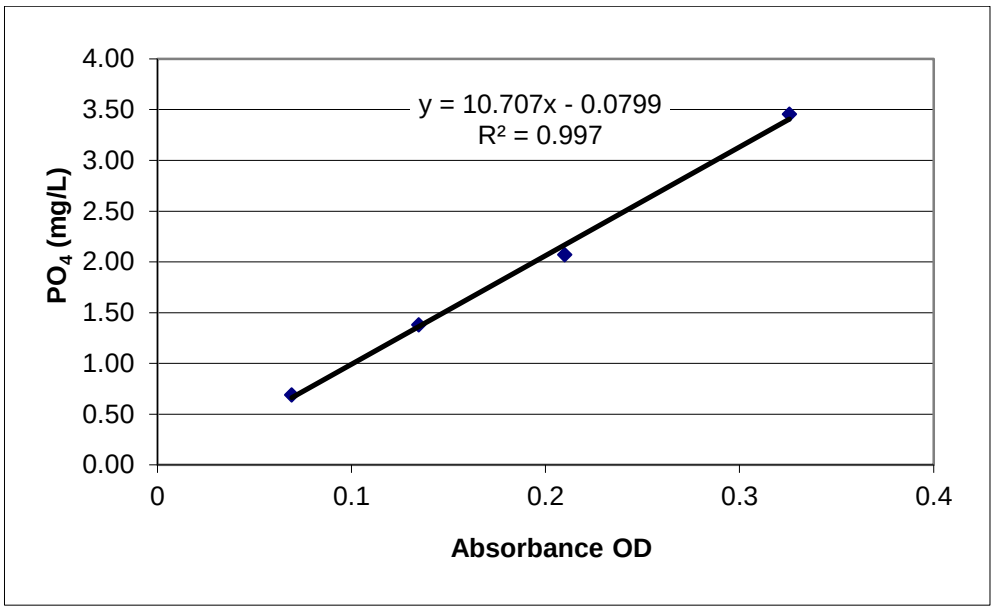


Figure A-2 Calibration curve for phosphate measurements using standards

A.1.2 Results for the uptake of ammonium and nitrogen in each set

Measurements were taken every two days during the experiment to measure both the ammonium and phosphate concentrations in each of the containers. The measurements were determined by the colorimetric method using reagent test kits (supplied by Spectroquant), the method is described in chapter 5 of the thesis. Tables A-3, A-4 and A-5 display the absorbance values, dilution rates and ammonium concentrations for set A, B and C respectively and for each container over the period of the experiment. Tables A-6, A-7 and A-8 display the absorbance values, dilution rates and phosphate concentrations for set A, B and C respectively for each container over the period of the experiment.

Table A-3 Absorbance values and ammonium concentrations for each container in set A (standard deviation in parentheses)

Day	Absorbance				Dilution	Ammonium (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.315	0.309	0.310	0.31	50	23.32	22.84	22.92	23.00	23.02 (0.26)
2	0.312	0.314	0.359	0.244	25	11.54	11.62	13.45	8.77	12.20 (1.08)
4	0.355	0.304	0.261	0.685	10	5.32	4.49	3.79	10.69	4.53 (0.77)
6	0.605	0.266	0.271	0.828	3.33 blank: 5	3.13	1.29	1.32	6.51	1.91 (1.05)
8	0.210	0.106	0.089	0.796	3.33	0.99	0.42	0.33	4.16	0.58 (0.36)

Table A-4 Absorbance values and ammonium concentrations for each container in set B (standard deviation in parentheses)

Day	Absorbance				Dilution	Ammonium (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.199	0.211	0.189	0.195	50	13.88	14.86	13.07	13.56	13.94 (0.90)
2	0.251	0.228	0.222	0.205	25	9.06	8.12	7.88	7.19	8.35 (0.62)
4	0.295	0.176	0.207	0.503	10	4.34	2.40	2.91	7.72	3.22 (1.00)
6	0.378	0.094	0.090	0.916	3.33	1.90	0.36	0.33	4.82	0.86 (0.90)
8	0.083	0.086	0.071	0.744	3.33	0.30	0.31	0.23	3.88	0.28 (0.04)

Table A-5 Absorbance values and ammonium concentrations for each container in set (standard deviation in parentheses)

Day	Absorbance				Dilution	Ammonium (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.118	0.132	0.133	0.112	50	7.29	8.43	8.51	6.80	8.08 (0.68)
2	0.142	0.161	0.115	0.156	25	4.62	5.40	3.52	5.19	4.51 (0.94)
4	0.150	0.089	0.064	0.291	10	1.98	0.99	0.58	4.27	1.18 (0.72)
6	0.079	0.089	0.097	0.278	1	0.08	0.10	0.11	4.06	0.10 (0.01)
8	0.133	0.114	0.110	0.267	1	0.17	0.14	0.13	3.88	0.15 (0.02)

Table A-6 Absorbance values and phosphate concentrations for each container in set A (standard deviation in parentheses)

Day	Absorbance				Dilution	Phosphate (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.611	0.613	0.598	0.586	10	41.70	41.84	40.82	40.00	41.45 (0.55)
2	0.569	0.375	0.387	0.513	10	38.85	25.65	26.47	35.04	30.32 (7.39)
4	0.416	0.398	0.308	0.457	10	28.44	27.22	21.10	31.23	25.58 (3.94)
6	0.476	0.342	0.364	0.459	10	32.52	23.41	24.90	31.37	26.95 (4.89)
8	0.334	0.277	0.223	0.498	10	22.86	18.99	18.99	34.02	19.06 (3.77)

Table A-7 Absorbance values and phosphate concentrations for each container in set B (standard deviation in parentheses)

Day	Absorbance				Dilution	Phosphate (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.415	0.415	0.393	0.381	10	28.37	28.37	26.88	26.06	27.87±0.86
2	0.32	0.263	0.273	0.333	10	21.91	18.04	18.72	22.80	19.55±2.07
4	0.3	0.224	0.183	0.369	10	20.55	15.38	12.60	25.24	16.18±4.04
6	0.235	0.281	0.353	0.549	5	8.07	9.63	12.08	18.74	9.92±2.02
8	0.278	0.212	0.122	0.487	5	9.53	7.28	4.22	16.63	7.01±2.66

Table A-8 Absorbance values and phosphate concentrations for each container in set C (standard deviation in parentheses)

Day	Absorbance				Dilution	Phosphate (mg/L)				Mean (mg/L)
	1	2	3	4		1	2	3	4	
0	0.286	0.218	0.193	0.238	10	19.60	14.98	13.28	16.34	15.95 (3.27)
2	0.153	0.142	0.118	0.169	10	10.56	9.81	8.17	11.64	9.51 (1.22)
4	0.291	0.129	0.154	0.285	5	9.97	4.46	5.31	9.77	6.58 (2.97)
6	0.097	0.123	0.069	0.417	3.33	2.25	2.84	1.61	9.50	2.23 (0.61)
8	0.089	0.065	0.058	0.356	3.33	2.07	1.52	1.36	8.12	1.65 (0.37)

For each dilution set, a blank container without algal biomass was used to check the ammonium and phosphate removal. The results from the concentration measurements are displayed in Figure A-3 and Figure A-4 below for ammonium and phosphate respectively.

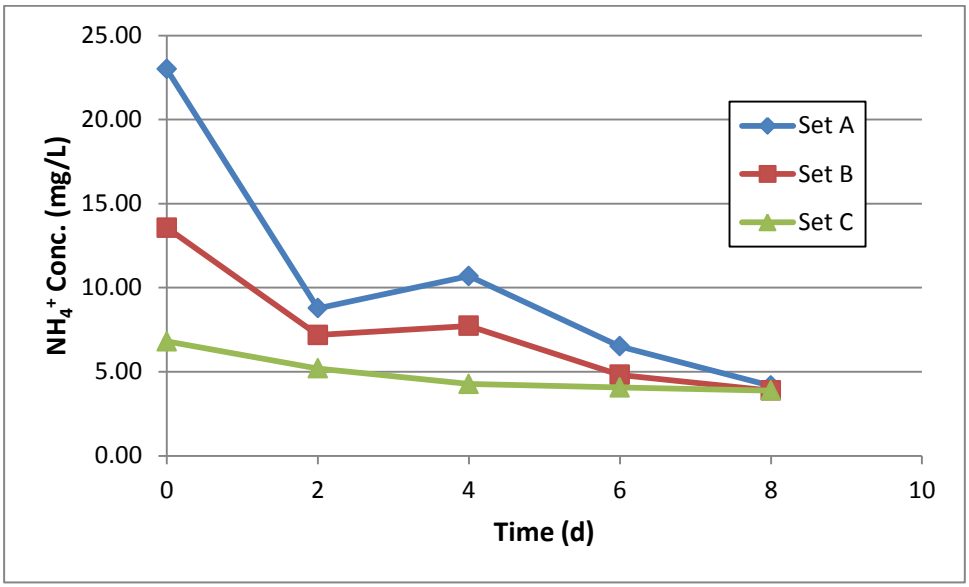


Figure A-3 Ammonium concentrations of the blank container for each set over time

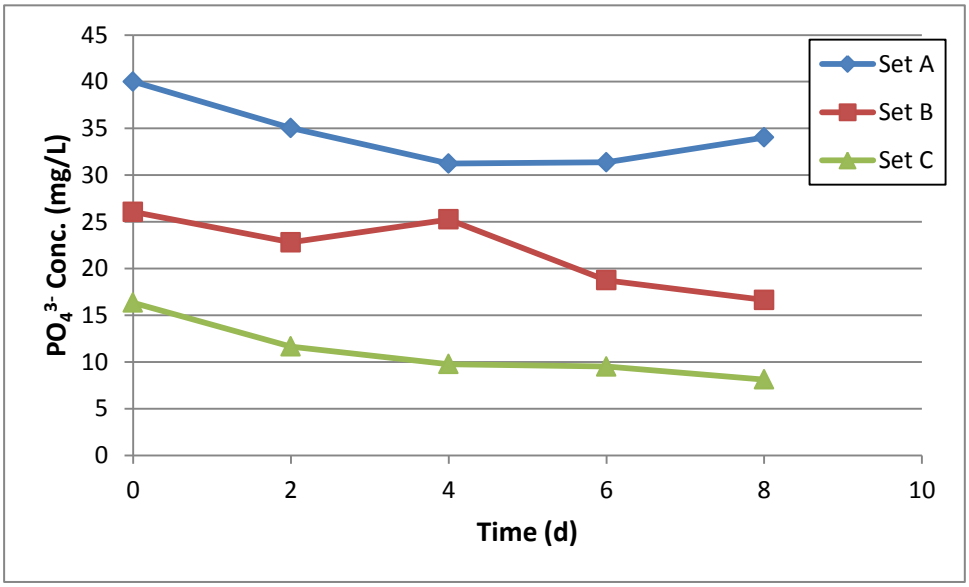
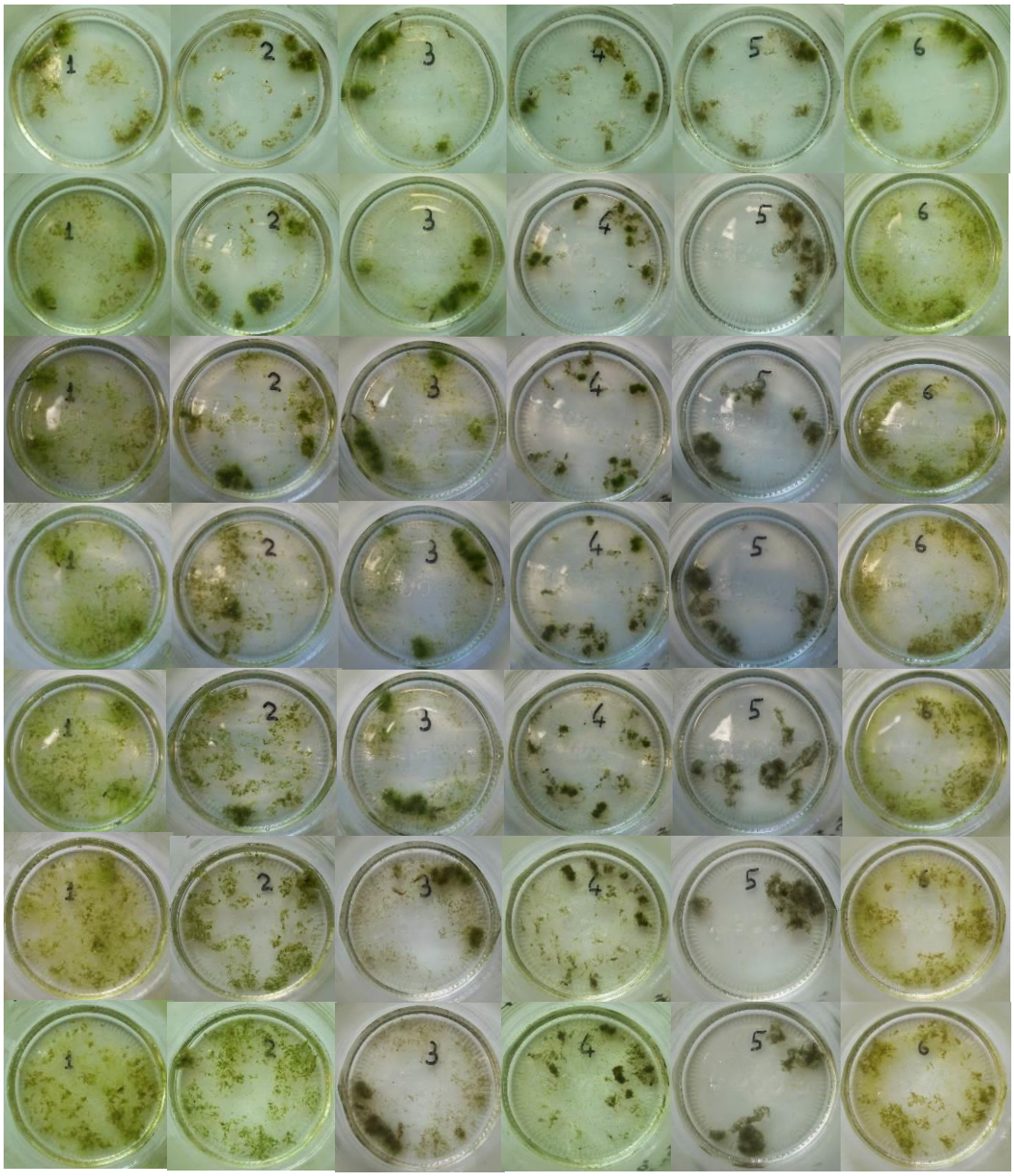


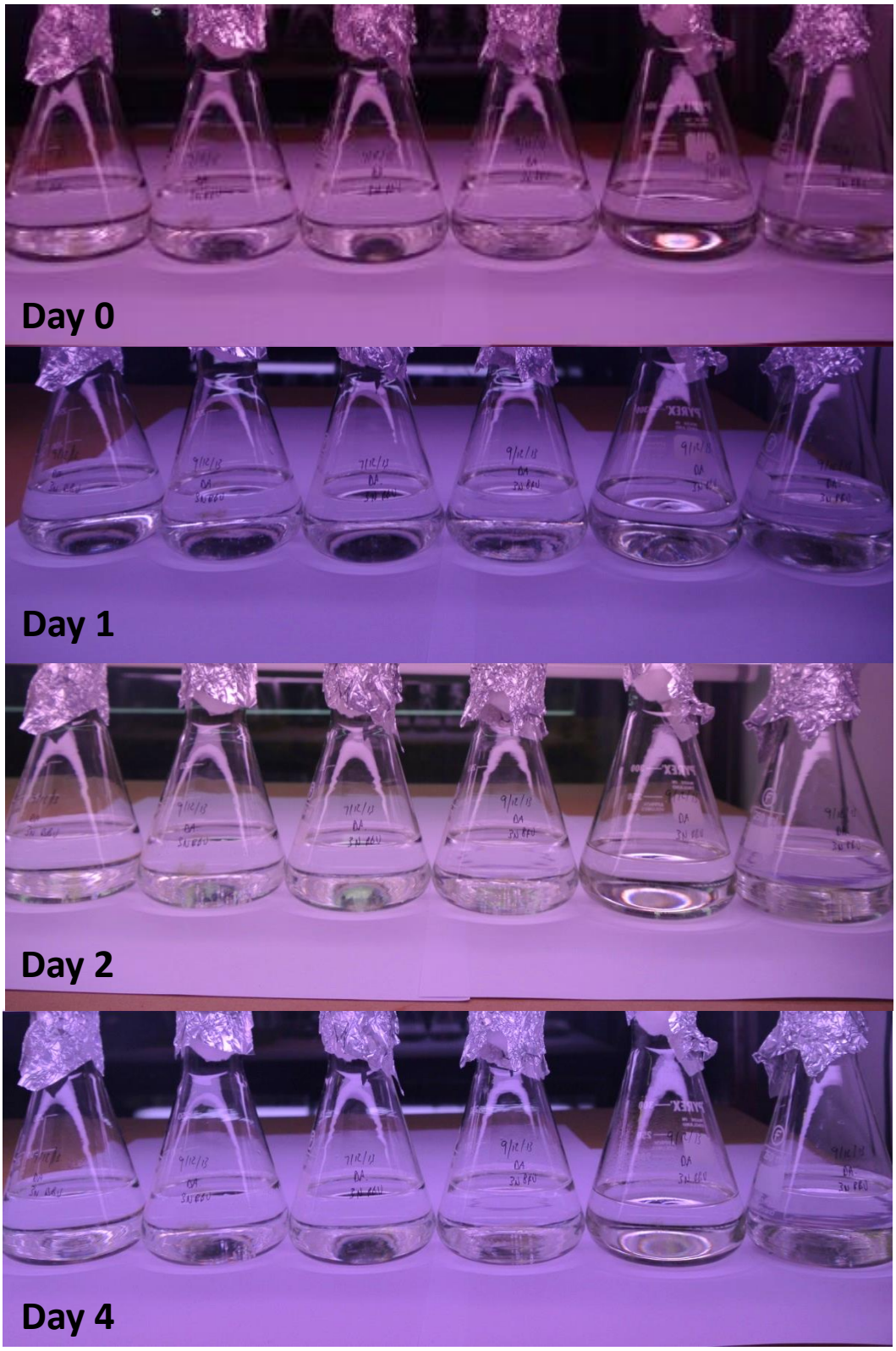
Figure A-4 Phosphate concentrations of the blank container for each set over time

A.2 Experimental photographs



1	2	3	4	5	6
Jaworski's medium	BG-11	Soil+ Water	Pond Water + Nutrients	Pure Water + Nutrients	Pond Water

Figure A-5 Photographs displaying the growth of the algal biomass in Winchester flasks and the media types


Day 0
Day 1
Day 2
Day 4

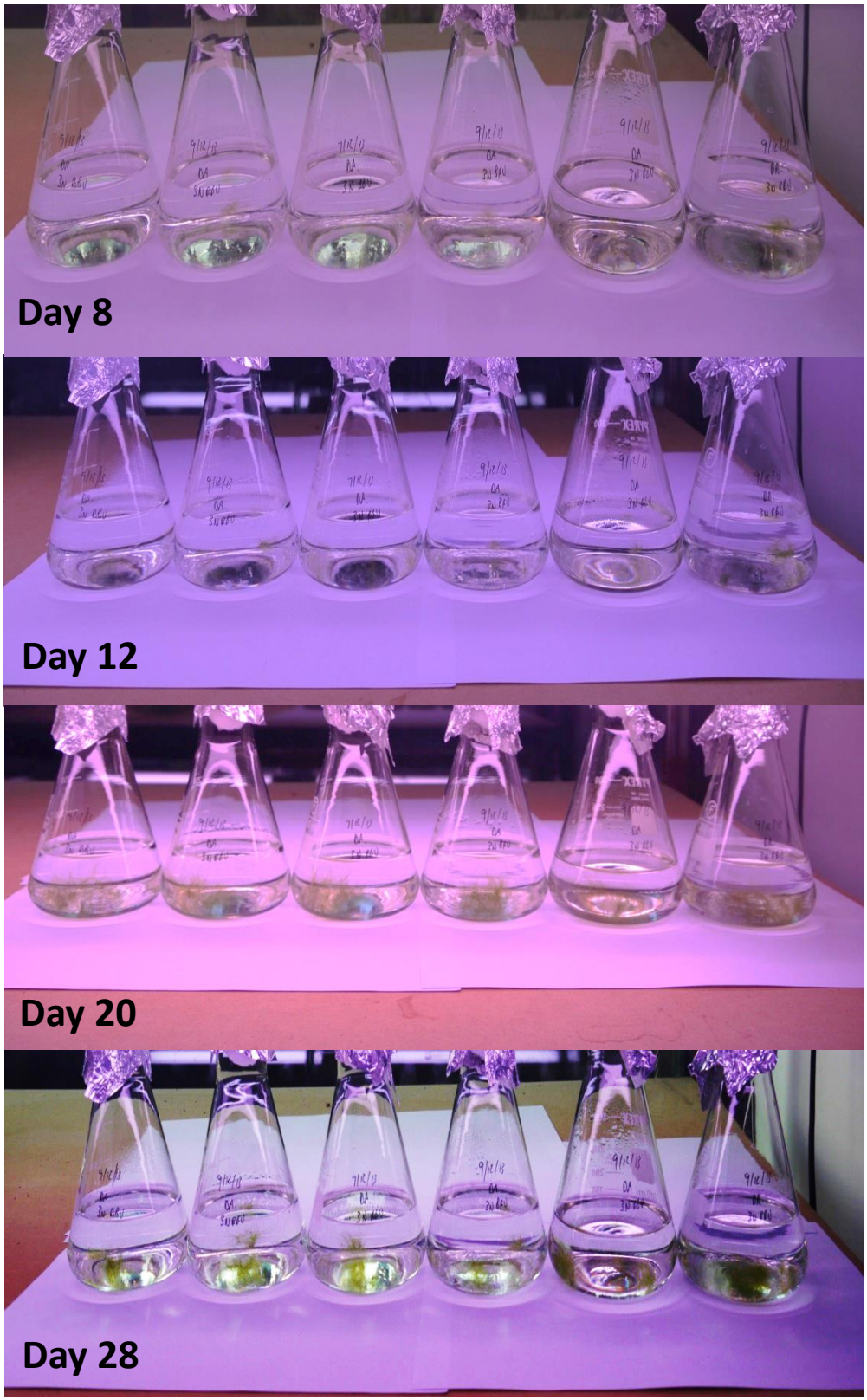


Figure A-5 Photographs displaying the growth of the *Spirogyra* algal biomass in Erlenmeyer flasks cultivated using Bold Basal Medium with 3 fold nitrogen and vitamins

A.3 Conversion of wild freshwater algal biomass to bioethanol and comparison with alternative biomass types

A.3.1 Ethanol calibration curve

The concentrations of ethanol were calculated using a gas chromatograph with flame-ionisation detector. A calibration curve was produced based on a set of standards produced with varying concentrations of ethanol from 0.2 to 5 g/L. 0.2 ml of each standard was added to a sample container and 1.8 ml of 1 g/L methanol standard was added. The containers were then capped and run through the GC. Using Chromquest software the areas of both the ethanol and methanol were calculated from the chromatographs produced. The values of the area for each concentration are displayed as Figure A-9.

Table A-9 Values of area for ethanol and methanol determined by gas chromatography and the corresponding amount ratio and R ratio

Ethanol (g/L)	Methanol (g/L)	Amount Ratio	Ethanol	Methanol	R Ratio
0.02	0.9	0.022	59575	3374542	0.017654
0.05	0.9	0.056	129165	3465880	0.037268
0.1	0.9	0.11	299443	3457584	0.086605
0.2	0.9	0.22	602760	3212402	0.187635
0.5	0.9	0.56	1489512	3033893	0.490957

The calibration curve calculated as a result of the areas determined for ethanol and methanol standards is displayed as figure A-6. The formula for calculation of the amount ratio is displayed on the graph.

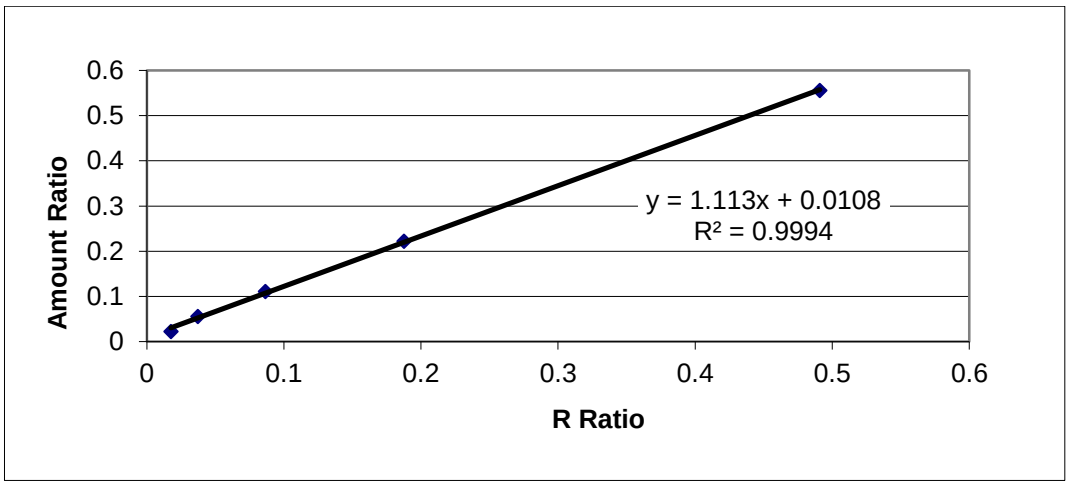


Figure A-6 The calibration curve for ethanol and methanol standards using GC-FID

A.3.2 Results for biomass fermentation

Following addition of yeast to each of the flasks, 0.2 ml samples of each flask were extracted with a pipette and placed in a sample container. 1.8 ml of 1 g/L methanol standard was also added to the container. Each sample was then run through the gas chromatograph and the areas were determined by the Chromquest software. The results for each sample are displayed in Figure A-10 and figure A-11 for areas of ethanol and methanol respectively for each sample time. Two samples were taken from each flask meaning a total of four samples for each biomass type. Where samples are not included there was no peak recorded by the gas chromatograph.

Table A-10 Values of area for ethanol of each sample flask and duplicate

Biomass	3h	6h	12h	24h
Algae 1 i	974026	994311	789220	489914
Algae 1 ii	1066688	1093010	852565	550502
Algae 2 i	1027254	1049634	782090	362217
Algae 2 ii	1045270	1013009	762751	440350
Seaweed 1 i	236673	111774	11965	-
Seaweed 1 ii	-	202026	13711	-
Seaweed 2 i	233932	151078	-	-
Seaweed 2 ii	239949	157087	-	-
Willow 1 i	449336	342004	131074	13464
Willow 1 ii	418303	323528	137678	9098
Willow 2 i	490689	500944	328833	-
Willow 2 ii	481513	404254	230779	-
MSW 1 i	536121	384623	204910	77585
MSW 1 ii	591687	375666	185434	89245
MSW 2 i	547227	442378	226514	94644
MSW 2 ii	577329	409341	221919	96828
Alpha 1 i	1606645	1951309	1381235	1487888
Alpha 1 ii	1453764	1499969	1566488	1675034
Alpha 2 i	1537846	1557173	1257310	1389166
Alpha 2 ii	1623844	1543059	1223970	1477517

Table A-11 Values of area for methanol of each sample flask and duplicate

Biomass	3h	6h	12h	24h
Algae 1 i	2870024	2789555	2657319	2580151
Algae 1 ii	3129275	3017978	2713259	2545090
Algae 2 i	3025610	3063904	2619679	2576631
Algae 2 ii	3207085	3080297	2869959	2481373
Seaweed 1 i	3067014	2529084	2651624	-
Seaweed 1 ii	-	2918236	2945934	-
Seaweed 2 i	3528904	2912272	-	-
Seaweed 2 ii	3106225	3185390	-	-
Willow 1 i	3408714	2756446	2663068	2572380
Willow 1 ii	3188850	2888355	2777416	2490060
Willow 2 i	3136641	3104092	3734462	-
Willow 2 ii	3283209	2859734	2879805	-
MSW 1 i	3191388	2590258	2339656	2636956
MSW 1 ii	3383594	2696833	2460262	2641700
MSW 2 i	3141448	2681013	2397327	2878568
MSW 2 ii	3114717	2682159	2323505	2609483
Alpha 1 i	3224024	3061019	2422668	2749288
Alpha 1 ii	3325318	2769339	2505929	2844987
Alpha 2 i	3260881	2817704	2400149	2778598

Alpha 2 ii	2960040	3020251	2547813	3077919

The corresponding R ratios (ethanol area/methanol area) are displayed in Table A-12.

Table A-12 Response ratio values for each sample flask and duplicate

	3h	6h	12h	24h
Algae 1 i	0.339379	0.356441	0.296999	0.189878
Algae 1 ii	0.340874	0.362166	0.314222	0.2163
Algae 2 i	0.33952	0.342581	0.298544	0.140578
Algae 2 ii	0.325925	0.328867	0.265771	0.177462
Seaweed 1 i	0.077167	0.044195	0.004512	-
Seaweed 1 ii	-	0.069229	0.004654	-
Seaweed 2 i	0.06629	0.051876	-	-
Seaweed 2 ii	0.077248	0.049315	-	-
Willow 1 i	0.13182	0.124074	0.049219	0.005234
Willow 1 ii	0.131177	0.112011	0.049571	0.003654
Willow 2 i	0.156438	0.161382	0.088054	-
Willow 2 ii	0.146659	0.141361	0.080137	-
MSW 1 i	0.16799	0.148488	0.087581	0.029422
MSW 1 ii	0.174869	0.139299	0.075372	0.033783
MSW 2 i	0.174196	0.165004	0.094486	0.032879
MSW 2 ii	0.185355	0.152616	0.09551	0.037106
α-cellulose 1 i	0.498335	0.63747	0.57013	0.54119
α-cellulose 1 ii	0.43718	0.541634	0.625113	0.588767
Alpha 2 i	0.471604	0.552639	0.523847	0.499952
Alpha 2 ii	0.548589	0.510904	0.4804	0.480038

The equivalent amount ratios were calculated based on the equation determined by the calibration curve:

$$A\ Ratio = 1.113 \times R\ Ratio + 0.00108$$

The calculated values of amount ratio are displayed in Table A-13.

Table A-13 Amount ratios calculated from the response ratio using the calibration curve

	3h	6h	12h	24h
Algae 1 i	0.388529	0.407519	0.34135943	0.222134
Algae 1 ii	0.390193	0.413891	0.36052881	0.251541
Algae 2 i	0.388685	0.392092	0.34307971	0.167263
Algae 2 ii	0.373555	0.376829	0.30660278	0.208315
Seaweed 1 i	0.096687	0.05999	0.01582222	-
Seaweed 1 ii	-	0.087852	0.01598014	-
Seaweed 2 i	0.084581	0.068538	-	-
Seaweed 2 ii	0.096777	0.065687	-	-
Willow 1 i	0.157515	0.148895	0.06558094	0.016626
Willow 1 ii	0.1568	0.135468	0.06597201	0.014867
Willow 2 i	0.184915	0.190418	0.10880371	-
Willow 2 ii	0.174032	0.168134	0.09999251	-
MSW 1 i	0.197773	0.176067	0.10827793	0.043547
MSW 1 ii	0.20543	0.16584	0.09468864	0.048401
MSW 2 i	0.20468	0.19445	0.11596299	0.047394
MSW 2 ii	0.2171	0.180662	0.11710313	0.052099
α-cellulose 1 i	0.565447	0.720305	0.64535437	0.613145
α-cellulose 1 ii	0.497382	0.613639	0.70655042	0.666097
α-cellulose 2 i	0.535696	0.625887	0.59384132	0.567247
α-cellulose 2 ii	0.621379	0.579436	0.54548548	0.545082

Using the values of the amount ratio determined for each sample, the ethanol concentration was calculated based on the ratio of ethanol to methanol (0.1/0.9). The concentrations of ethanol were calculated by multiplying the amount ratios by 0.9 and 10. The calculated values of ethanol concentration are displayed in Table A-20.

Table A-14 Ethanol concentrations calculated from the amount ratio

	3h	6h	12h	24h
Algae 1 i	3.49676	3.667667	3.072235	1.999208
Algae 1 ii	3.511733	3.72502	3.244759	2.263873
Algae 2 i	3.498168	3.52883	3.087717	1.505367
Algae 2 ii	3.361993	3.391464	2.759425	1.874839
Seaweed 1 i	0.870184	0.539906	0.1424	-
Seaweed 1 ii	-	0.790665	0.143821	-
Seaweed 2 i	0.76123	0.616845	-	-
Seaweed 2 ii	0.870991	0.591187	-	-
Willow 1 i	1.417639	1.340052	0.590228	0.14963
Willow 1 ii	1.411198	1.219216	0.593748	0.133799
Willow 2 i	1.664237	1.713762	0.979233	-
Willow 2 ii	1.566286	1.51321	0.899933	-
MSW 1 i	1.779955	1.584607	0.974501	0.391922
MSW 1 ii	1.848867	1.492558	0.852198	0.435606
MSW 2 i	1.842119	1.750046	1.043667	0.426547
MSW 2 ii	1.953903	1.625957	1.053928	0.468893
α-cellulose 1 i	5.089025	6.482741	5.808189	5.518303
α-cellulose 1 ii	4.476437	5.522751	6.358954	5.994877
α-cellulose 2 i	4.821262	5.632984	5.344572	5.105221
α-cellulose 2 ii	5.592411	5.214928	4.909369	4.905737

Table A-21 displays the mean ethanol concentrations for each sample flask with the values of standard deviation and Table A-22 displays the mean ethanol concentration for each biomass type with the values of standard deviation.

Table A-15 Mean ethanol concentrations from each sample flask

	Ethanol concentration (g/L)			
	3h	6h	12h	24h
Algae 1	3.50±0.01	3.70±0.04	3.16±0.12	2.13±0.18
Algae 2	3.43±0.10	3.46±0.10	2.92±0.23	1.69±0.26
Seaweed 1	0.87	0.54±0.18	0.14±0.001	-
Seaweed 2	0.82±0.08	0.60±0.02	-	-
Willow 1	1.41±0.005	1.28±0.09	0.59±0.002	0.14±0.01
Willow 2	1.62±0.07	1.61±0.14	0.94±0.06	-
MSW 1	1.81±0.05	1.54±0.07	0.91±0.09	0.41±0.03
MSW 2	1.90±0.08	1.69±0.09	1.05±0.007	0.45±0.03
α-cellulose 1	4.78±0.43	6.00±0.68	6.08±0.39	5.76±0.34
α-cellulose 2	5.21±0.55	5.42±0.30	5.13±0.31	5.01±0.14

Table A-16 Mean ethanol concentrations for each biomass type

	Ethanol concentration (g/L)			
	3h	6h	12h	24h
Algae	3.47±0.05	3.58±0.17	3.04±0.17	1.91±0.32
Seaweed	0.84±0.04	0.57±0.05	-	0.85
Willow	1.51±0.14	1.45±0.24	0.77±0.25	-
MSW	1.86±0.06	1.61±0.11	0.98±0.10	0.07±0.02
α-cellulose	4.99±0.30	5.71±0.42	5.61±0.69	5.38±0.54

Appendix B: A life cycle assessment comparison of algal cultivation and conventional methods for nutrient removal of wastewater

B.1 Wastewater characteristics

The influent wastewater characteristics were taken as the average annual values from the 2010 Haifa WWTP report. The values are displayed as table B-1.

Table B-1 Haifa WWTP influent wastewater characteristics

Characteristic	Value
Flow	120,000 m^3
COD	1.192 g/L
BOD	0.469 g/L
TSS	0.644 g/L
VSS	0.556 g/L
TN	0.105 g/L
NH_4	0.076 g/L
TP	0.0049 g/L

B.2 Israel national electricity grid

The national electricity grid was modelled on data taken from the Ministry of National Infrastructures. The proportion of each electricity source to the national grid was reported as 65% coal, 33% natural gas and 2% liquid fuel [207]. The impacts of the electricity generated from the national grid were calculated based on the impacts for each of the sources using data from Ecoinvent [106] and the above proportions.

B.3 Scenario 1

Scenario 1 assumed a similar set-up to the existing system but with the replacement of the activated sludge process with an enhanced nutrient removal process, the A_2O process. The A_2O process is a method for effective removal of both nitrogen and phosphorous using different processing conditions.

B.3.1 Primary clarification

The energy consumption for the primary clarification was a result of scraping the sludge to be sent to the anaerobic digester. The energy consumed in the process was calculated based on work conducted by Graif [246] who collected the energy consumption data of wastewater treatment plants in Israel. A relationship was determined to calculate the energy required for sludge scraping which was:

$$Energy_{scraper} = 0.0039 \times Q + 16.72 \quad \text{(B.1-1)}$$

For an influent flow (influent flow to WWTP and recycled effluent) of 124, 404 m^3, the energy of the scraper was calculated as:

$$E_{scraper} = 0.0039 \times 124{,}404 + 16.72 = 502\ kWh \quad \text{(B.1-2)}$$

The mass of sludge entering the primary clarification was calculated using the concentration of total suspended solids (TSS). The TSS concentration entering the WWTP was 0.644 kg/m^3 however the recycled effluent from the subsequent thickening and de-watering processes had higher TSS concentrations. The combined TSS was calculated to be 0.707 kg/m^3. For a flow of 124,435 m^3, the total sludge entering the clarifier was 87,997 kg. Primary clarification was assumed to remove 55% of this sludge from the wastewater equalling 48,399 kg which was sent to the anaerobic digestion facility. The density of the sludge was assumed to be 1000 kg/m^3 and with a calculated TSS of 37 kg/m^3, the flow as calculated to be:

$$Q_{primary\ sludge} = 48{,}399\ kg \times \frac{1}{0.037\ kg/m^3} = 1{,}308\ m^3 \quad \text{(B.1-3)}$$

B.3.2 A_2O Process

The next stage of the system was the A_2O process for removal of BOD, COD and nutrients. In the existing set up this was conducted by mechanical aeration, however to achieve adequate nitrogen (N) and phosphorous (P) removal, a more advanced method of removal is necessary. Energy was required for mixing in the anoxic and anaerobic tanks, sludge pumping, water pumping and air diffusion in the aerobic tank as well as materials for construction.

The power requirement of the mixing was calculated using the equation from Metcalf and Eddy [200] for a mechanical mixing device. Typical parameters for the mixing were used for rapid mixing and the dynamic viscosity for a temperature of 20°C. The flow into the tanks was calculated to be 123,152 m^3. The energy required for each tank was calculated as:

$$E_{mixing} = 1000^2 \left(\frac{l}{s}\right)^2 \times 0.001 pa\ s \times 2.3 \times 10^{-4} d \times 123{,}096 m^3 \times 24h \times \frac{1}{1000}\frac{kW}{W} = 679.5\ kWh \quad \text{(B.1-4)}$$

B.3.3 Aeration

The power requirements of air diffusion in the aerobic tank depended upon the required production of bacterial biomass and the required reduction in Chemical Oxygen Demand (COD) and Nitrified ammonia concentration (NO_x).

The COD uptake of the previous processes were first calculated. The COD input to the anaerobic tank was calculated based on the relationship between BOD and COD, using assumptions from Metcalf and Eddy [200] which were:

bCOD = 1.6BOD

bCOD = 0.68COD

The BOD entering the anaerobic tank was calculated assuming that 35% of the BOD was removed from the primary clarification process based on data from Chagnon *et al.* [247].The BOD entering the anaerobic tank was therefore calculated as:

$$Q_{BOD} = \left(120000\ m^3 \times 0.435\frac{kg}{m^3} + 4435\ m^3 \times 1.415\frac{kg}{m^3}\right) \times 0.65 = 40660\ kg \quad \text{(B.1-5)}$$

The COD entering the anaerobic tank was calculated as:

$$Q_{COD} = 40660\ kg \times \frac{1.6}{0.68} = 95670\ kg \quad \text{(B.1-6)}$$

The mass of phosphorous entering the anaerobic tank was calculated assuming 20% was removed during primary clarification [247], the recycled effluent was assumed to

have a negligible phosphrous concentration. The phosphorous concentration was therefore calculated as:

$$Q_{TP} = \left(12000m^3 \times 0.0049\frac{kg}{m^3}\right) \times \frac{120000}{120000+4435} \times 0.8 = 470.4\,kg \quad \text{(B.1-7)}$$

The ratio of COD/P was therefore calculated as:

$$\frac{COD}{P} = \frac{95670}{470.4}\frac{kg}{kg} = 203.4 \quad \text{(B.1-8)}$$

The uptake of COD in the anaerobic tank was calculated based on a relationship developed by Punrattanasin [248]. The COD uptake in the anaerobic tank was measured for various COD/P ratios. The results are shown in table B-2. The experiments used a daily flow of 40 L, the COD uptake values were therefore multiplied by ratio of flow into the anaerobic tank in this study (123,096 m^3) by the volume in the experiment (0.04 m^3) to obtain the equivalent uptake rates (table 2). Using this data, a relationship was determined between the (COD/P) ratio to the COD uptake (Figure B-1).

Table B-2 COD uptake values for different COD/P ratio values from Punrattanasin [248] and equivalent values for this study

COD/P	COD uptake (g/day) (0.04 m^3)	COD uptake (kg/day) (123,152 m^3)
20	13,892	42,770
30	11,107	38,239
40	13,389	38,996
60	10,763	33,136

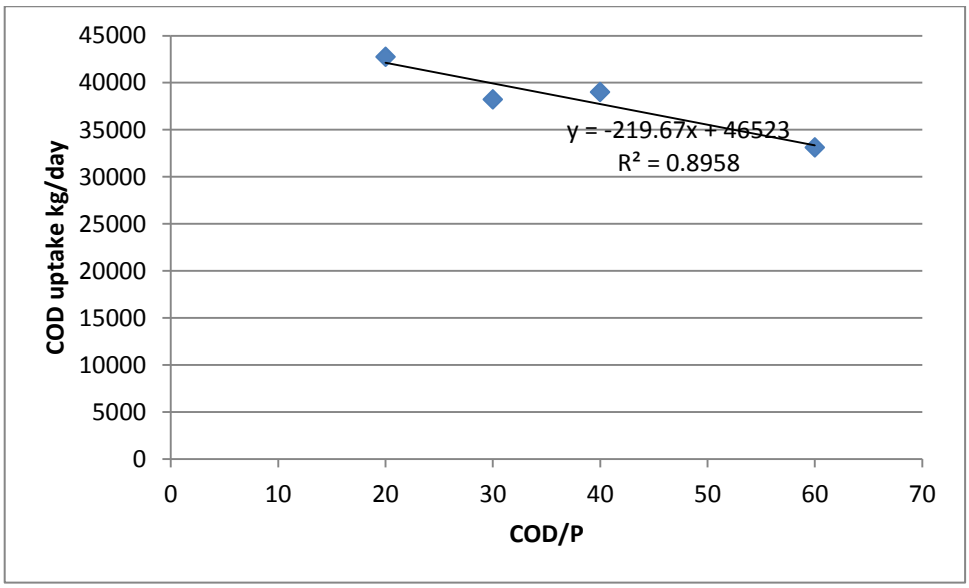


Figure B-1 Relationship between COD uptake and COD/P ratio

For the calculated COD/P value of 203.4, using the relationship determined in figure 1 the COD uptake was calculated:

$$Q_{COD\_anaerobic} = -219.7 \times 203.4 + 46{,}523 = 1{,}876\ kg \qquad \text{(B.1-9)}$$

According to [249] a COD/P greater than 35 is required for good removal of phosphorous (less than 1mg/L), therefore a COD/P ratio of 203.3 was assumed to provide sufficient P removal.

According to Randall *et al.* [249] the COD in the anoxic reactor is consumed for the oxidation of nitrate, to create cell energy and for cell growth. The equation for the COD uptake is therefore:

$$Q_{COD\_anoxic} = Q_{COD\_energy} + Q_{COD\_cell} \qquad \text{(B.1-10)}$$

Where:

Q_{COD_anoxic} = COD consumed in the anoxic process (kg/d)

Q_{COD_energy} = COD consumed for nitrate oxidation and for cell energy (kg/d)

Q_{COD_cell} = COD consumed during cell growth (kg/d)

For each kg of nitrate de-nitrified, 2.86 kg of COD is consumed. The equation for the COD amount consumed for energy production was therefore calculated as:

$$Q_{COD\_energy} = 2.86 \times Q_{NO3-N\_reduced} \quad \text{(B.1-11)}$$

The value of nitrate to be denitrified was determined by subtracting the required effluent NH_3 standard (0.0015 kg/m^3) from the influent NH_3 concentration (0.076 kg/m^3). The COD uptake for energy production was therefore calculated as:

$$Q_{COD\_energy} = 2.86 \times 0.0745 \frac{kg}{m^3} \times 123{,}096\ m^3 = 26{,}228\ kg \quad \text{(B.1-12)}$$

The proportion of the COD uptake for the cell growth was determined using a relationship with the total COD uptake in the anoxic tank:

$$Q_{COD\_energy} = \frac{1.42 \times Y}{1 + k_d SRT} \times Q_{COD\_anoxic} \quad \text{(B.1-13)}$$

Using values for a temperature of 20°C from Metcalf and Eddy [200] (Y = 0.4 g VSS/ g bCOD, k_d = 0.12 l/d, SRT = 10 days), the proportion of the total COD uptake was calculated as:

$$Q_{COD\_energy} = \frac{1.42 \times 0.4}{1 + 0.12 \times 10} \times Q_{COD\_anoxic} = 0.258 \times Q_{COD\_anoxic} \quad \text{(B.1-14)}$$

The COD uptake of the anoxic tank was therefore calculated as:

$$Q_{COD\_anoxic} = 26{,}228 \times \frac{1}{0.742} = 35{,}356\ kg \quad \text{(B.1-15)}$$

The power of the diffuser in the aerobic tank was calculated as a function of the flow of air required to produce sufficient microbial biomass to reduce bCOD and nutrients to the required levels. The equation used for the biomass productions was:

$$W_{Bio} = \frac{QY(S_{bCOD_0} - S_{bCOD})}{1 + k_d SRT} + \frac{f_d k_d QY(S_{bCOD_0} - S_{bCOD}) SRT}{1 + k_d SRT} + \frac{QY_n(NO_x)}{1 + k_{dn} SRT} \quad \text{(B.1-16)}$$

The $bCOD_0$ value was calculated by subtracting the COD uptake in the previous tanks from the COD value into the A_2O process:

$$S_{bCOD_0} = \frac{95{,}606 - 1{,}876 - 35{,}356}{123{,}096} \frac{kg}{m^3} \times 0.68 = 0.323 \frac{kg}{m^3} \quad \text{(B.1-17)}$$

The effluent discharge standard for BOD was 0.01 kg/m^3 equating to 0.016 kg/m^3 bCOD. Typical parameters were used from Metcalf and Eddy [200] for a temperature of 20°C (Y = 0.4 g VSS/ g bCOD, k_d = 0.12 l/d, SRT = 10 days, f_d = 0.15, Y_n = 0.12g VSS/g NH_4-N, k_{dn}=0.08 l/d). The mass of biomass was therefore calculated as:

$$W_{Bio} = \frac{123{,}096 \times 0.4 \times (0.323 - 0.016) \times (1 + 0.15 \times 0.12 \times 10)}{1 + 0.12 \times 10} + \frac{123{,}096 \times 0.12 \times 0.0745}{1 + 0.08 \times 10} = 8{,}593\ kg \quad \text{(B.1-18)}$$

The oxygen uptake rate was calculated using the following equation taken from Metclaf and Eddy [200]:

$$OUR = 123{,}096\ m^3 \times (0.331 - 0.016)\frac{kg}{m^3} - 1.42 \times 8{,}716 kg + 4.33 \times 123{,}096 m^3 \times 0.0745\frac{kg}{m^3} = 57{,}937\ kg \quad \text{(B.1-19)}$$

The mass of oxygen required also depends upon the oxygen transfer constants (table 1). The standard oxygen consumption was calculated as:

$$SOTR = \frac{57{,}397}{\frac{0.95 \times 14.552 - 2}{9.08} \times 0.5 \times 0.75} kg = 118{,}640\ kg \quad \text{(B.1-20)}$$

Considering an oxidation efficiency of 25% and an atmospheric oxygen content of 21%, the mass of air required for diffusion was calculated as:

$$W_{air} = \frac{133{,}362}{24 \times 3{,}600 \times 0.25 \times 0.21}\frac{kg}{s} = 26.2\frac{kg}{s} \quad \text{(B.1-21)}$$

The diffusers energy consumption was calculated using the equation for the power rating of an air blower from Metcalf and Eddy [200]:

$$P_{diffuser} = \frac{W_{air} \times R \times T_{env}}{M_{w_{air}} \times n \times e_{blower}} \times ((p_H / p_{H=0})^n - 1 \quad \text{(B.1-22)}$$

The values of the diffuser constants were assumed to be: R=8.314, M_{wair}=29.7, n=0.283 and e_{blower}=0.7. the depth of the tanks was assumed to be 10 m which equated to a pressure of 1496 mm Hg, the pressure at the surface was assumed to be 760 mm Hg. The energy of the diffuser was calculated as:

$$E_{diffuser} = \frac{26.2 \times 8.314 \times 293}{29.7 \times 0.283 \times 0.7} \times ((\frac{1496}{760})^{0.283} - 1) \times 24 = 35{,}919\ kWh$$

(B.1-23)

B.3.4 Pumping

Pumps are required within the A_2O process to recycle wastewater and sludge. The power consumption equation for a pump [250] was used for both wastewater and sludge recycling. The Total Dynamic Head (TDH) for sludge recycling was assumed to be 10 m with a pump efficiency of 60% and a recycling rate of 75%. The TDH for wastewater recycling was assumed to be 4 m with a pump efficiency of 80% and a recycling rate of 300%. The energy consumption of the sludge recycle pump was calculated as:

$$E_{sludge} = 123{,}096 m^3 \times 0.75 \times 9.81 \frac{g}{s^2} \times 1000 \frac{kg}{m^3} \times 10m \times \frac{1}{0.6} \times \frac{1}{1000} \frac{kW}{W} \times \frac{1}{60 \times 60} = 4{,}193\ kWh$$

(B.1-24)

The energy consumption of the wastewater recycle pump was calculated as:

$$E_{ww} = 123{,}096 m^3 \times 3 \times 9.81 \frac{g}{s^2} \times 1000 \frac{kg}{m^3} \times 4m \times \frac{1}{0.8} \times \frac{1}{1000} \frac{kW}{W} \times \frac{1}{60 \times 60} = 7{,}547\ kWh$$

(B.1-25)

B.3.5 A_2O Infrastructure

Extra infrastructure is necessary to allow for the A_2O process, this study considered the production of the concrete tanks necessary. The tank sizes depended upon the retention time of the wastewater. The retention times were calculated by taking the mean values of the ranges suggested by [200] for the anaerobic, anoxic and aerobic tanks. The retention times were 1 hour, 0.75 hours and 6 hours for anaerobic, anoxic and aerobic tanks respectively. For a detention time of 1 hour, the necessary volume for the anaerobic tank was calculated to be:

$$V_{anaerobic} = \frac{123{,}096\ m^3}{24\ h} \times 1h = 5{,}129\ m^3 \quad \text{(B.1-26)}$$

A fill volume of 2/3 was assumed, therefore the total volume was calculated to be 7,694 m^3. The tank wall was assumed to be constructed from 0.5 m concrete blocks with a 0.5 m floor slab. The height was assumed to be 6 m, therefore the area was calculated to be 1,282.3 m^2. Assuming the tank is a square, each length is 35.8 m. The mass of concrete block, assuming a density of 2,380 kg/m^3 and life span of 20 years, was calculated to be:

$$M_{block} = (36.8m \times 6m \times 0.5m \times 2 + 35.8m \times 6 \times 0.5m \times 2) \times 2{,}380 \frac{kg}{m^3} \times \frac{1}{20y \times 365d} = 142.1kg \quad \text{(B.1-27)}$$

Assuming a 20 year life span, the volume of the concrete slab was calculated as:

$$V_{slab} = 1{,}282.3m^2 \times 0.5m \times \frac{1}{20y \times 365d} = 0.0878m^3 \quad \text{(B.1-28)}$$

The anoxic tank had a detention time of 0.75 hours, the necessary tank volume was therefore calculated as:

$$V_{anoxic} = \frac{123{,}096\ m^3}{24\ h} \times 0.75h = 3{,}847\ m^3 \quad \text{(B.1-29)}$$

Assuming a fill volume ratio of 2/3, the total required volume was calculated to be 5,771 m^3. With a height of 6 m, the area was 962 m^2 equating to a length of 31 m for a square tank. The tank walls were assumed to be of 0.5 m thickness. The mass of concrete block was therefore calculated as:

$$M_{block} = (32m \times 6m \times 0.5m \times 2 + 31m \times 6 \times 0.5m \times 2) \times 2{,}380 \frac{kg}{m^3} \times \frac{1}{20y \times 365d} = 123.3kg \quad \text{(B.1-30)}$$

The volume of concrete slab was calculated as:

$$V_{slab} = 961.8m^2 \times 0.5m \times \frac{1}{20y \times 365d} = 0.0659m^3 \quad \text{(B.1-31)}$$

The aerobic tank was assumed to have a detention time of 6 hours, the required volume was calculated as:

$$V_{anoxic} = \frac{123{,}096\ m^3}{24\ h} \times 6h = 30{,}774\ m^3 \quad \text{(B.1-32)}$$

The total tank volume was calculated to be 46,161 m^3 for a fill volume ratio of 2/3. For a height of 10 m, the area was calculated to be 4,616 m^2. Assuming a square tank, the wall length was calculated to be 67.9 m. The mass of concrete block was therefore calculated as:

$$M_{block} = (68.9m \times 10m \times 0.5m \times 2 + 67.9m \times 6 \times 0.5m \times 2) \times 2{,}380\ \frac{kg}{m^3} \times \frac{1}{20y \times 365d} = 446.3kg \quad \text{(B.1-33)}$$

The volume of concrete slab was calculated as:

$$V_{slab} = 4{,}616m^2 \times 0.5m \times \frac{1}{20y \times 365d} = 0.316m^3 \quad \text{(B.1-34)}$$

B.3.6 Secondary clarification

The same relationship for the energy consumption of the sludge scraper was used for the secondary clarification as for the primary clarification. The flow into the secondary clarifier was 123,096 m^3. the energy requirement of the sludge scraper was calculated as:

$$E_{sc} = 0.0039 \times 123{,}096m^3 + 16.72 = 497\ kWh \quad \text{(B.1-35)}$$

The mass of sludge obtained from the secondary clarification was calculated based on the equation for solids production during biological treatment in Metcalf and Eddy [200]. The variable values used are as follows: Y=0.4 g VSS/gbCOD, k_d=0.12 L/d, f_d=0.15, SRT=10 d. The initial BOD concentration was calculated to be 0.33 kg/m^3, the final BOD concentration was 0.01 kg/m^3. The initial NH_4 concentration was calculated to be 0.062 kg/m^3 and the final concentration, 0.0015 kg/m^3. The initial TSS concentration was 0.322 kg/m^3 and assuming 10% of volatile suspended solids was non-biodegradable, the bVSS concentration was calculated to be 0.246 kg/m^3. The sludge mass was calculated as:

$$M_{sludge} = 123{,}096m^3 \times (0.4\frac{gVSS}{gbCOD} \times 1.6 \times \left(0.330\frac{kg}{m^3} - 0.010\frac{kg}{m^3}\right) \times \frac{1+0.12\frac{L}{d}\times 0.15\times 10\ d}{0.85\times\left(1+0.12\frac{L}{d}\times 10\ d\right)} +$$

$$\left(0.062\frac{kg}{m^3} - 0.0015\frac{kg}{m^3}\right) \times \frac{0.12\frac{L}{d}}{1+0.08\times 10d} + \left(0.322\frac{kg}{m^3} - 0.246\frac{kg}{m^3}\right)) = 25{,}717\ kg \quad \text{(B.1-36)}$$

The solids content of the secondary sludge was assumed to be 0.8%. The flow sent to the anaerobic digestion facility was therefore calculated as:

$$Q_{secondary\ sludge} = 25{,}717\ kg \times \frac{1}{0.008\times 1000\ kg/m^3} = 3{,}215\ m^3 \quad \text{(B.1-37)}$$

B.3.7 Gravity belt thickening

After scraping, both the primary and secondary sludge was sent to the gravity belt thickener prior to anaerobic digestion to reduce the water content. The energy consumption of the gravity belt thickener (GBT) was based on values obtained from GBT thickeners produced by Ecomacchine [251]. The relationship of power to flow rate is displayed as figure 1. The following equation was determined from the data:

$$P = 0.0004 \times Q + 0.825 \quad \text{(B.1-38)}$$

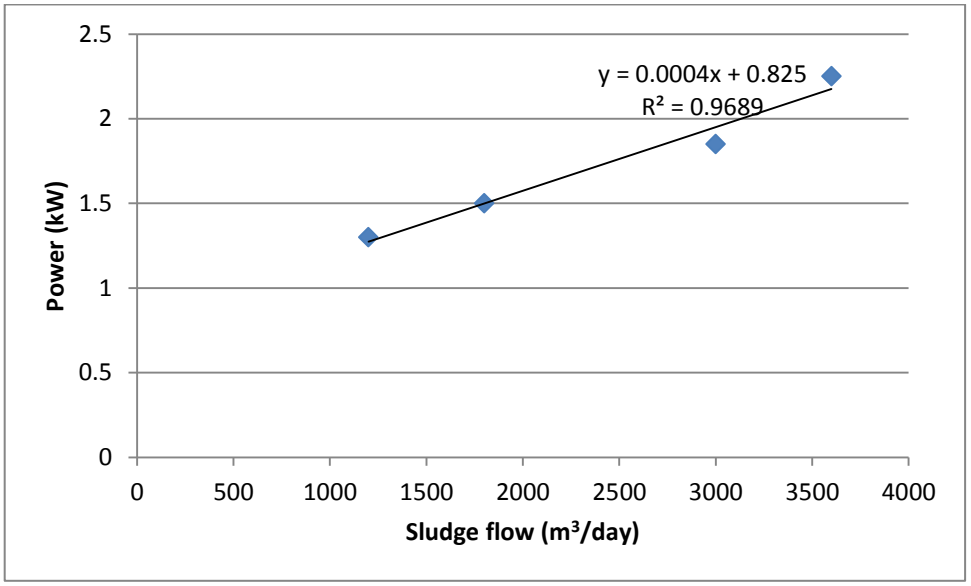


Figure B-1. Relationship of GBT power rating to sludge flow

The total sludge flow into the GBT was calculated as 4,522 m^3, the energy consumption of the gravity belt thickener was therefore calculated as:

$$E_{GBT} = (0.0004 \times 4{,}522 + 0.825) \times 24 = 63\ kWh \quad \text{(B.1-39)}$$

It was assumed that the gravity belt thickening process recovered 90% of the total solids and produced a concentrate with a total solids concentration of 5%. The mass of sludge recovered from the thickener was therefore calculated as:

$$M_{sludge} = 0.9 \times 74{,}116kg = 66{,}704kg \quad \text{(B.1-40)}$$

The corresponding flow was based on a density of 1000 kg/m^3 and was calculated as:

$$Q_{sludge} = 66{,}704kg \times \frac{1}{1000\frac{kg}{m^3} \times 0.05} = 1{,}334m^3 \quad \text{(B.1-41)}$$

The flow of effluent returned to the primary clarifier was calculated as:

$$Q_{return} = 4{,}497m^3 - 1{,}334m^3 = 3{,}163m^3 \quad \text{(B.1-42)}$$

B.3.8 Anaerobic digester

The total flow of sludge entering the anaerobic digester was calculated to be 1,334 m^3. The main energy consumption in the anaerobic digester is mixing and heating. Mixing was assumed to be mechanical, the energy consumption was based on the equation for mechanical mixing from Metcalf and Eddy [200]. An average power rating of 0.0065 kW/m^3 was used with an SRT of 10 days which is recommended for an operating temperature of 35°C [200]. The electricity consumed for mixing was therefore calculated as:

$$E_{mixing} = 1{,}334m^3 \times 10d \times 0.0065\ kw/m^3 \times 24h = 2{,}081\ kWh \quad \text{(B.1-43)}$$

Heating was considered necessary to increase the sludge temperature to the temperature of the digester which was assumed to operate at a mesophilic temperature of 35°C, the environmental temperature was assumed to be 20°C. The heat capacity of sludge was assumed to be the same as water, 4.18 kJ/kg.°C. The flow entering the anaerobic digester was 1,334 m^3 made up of 5% total solids, assuming that the density

is the same as the density of water, the mass entering the digester was 1,343 t. Using these values, the energy required to heat the sludge was calculated as:

$$E_{heat} = 4.18 \frac{MJ}{t℃} \times 1{,}343\ t \times (35 - 20)℃ = 83{,}647\ MJ \quad \text{(B.1-44)}$$

The heat lost through the walls, roof and floor of the digester was assumed to be 10% of the total heat required to heat the sludge as the environmental temperature in Israel is similar to the mesophilic temperature. The extra heat required to account for the heat loss was therefore calculated as 8,365 MJ. The existing infrastructure was assumed to be sufficient for the anaerobic digestion and therefore no extra materials were necessary.

The volatile solids reduction during digestion was calculated by using the equation for volatile solids reduction in Metcalf and Eddy [200]:

$$V_d = 13.7 \times \ln(10d) + 18.9 = 50.4\% \quad \text{(B.1-45)}$$

The mass of sludge released from the anaerobic digester was therefore calculated as:

$$M_{sludge} = 66704\ kg \times (1 - 0.504) = 33{,}059 kg \quad \text{(B.1-46)}$$

B.3.9 Sludge dewatering

After digestion the sludge was assumed to be dewatered and the solids disposed of. Sludge dewatering was carried out using centrifugation, the energy consumption of the centrifuge units were calculated using data from Culp [252] for different sludge flows. A relationship was determined by Kostovetsky [253] which was:

$$E_{dewatering} = 1.0054Q \quad \text{(B.1-47)}$$

The outflow of the digester was assumed to be the same as the influent, 1,334 m^3. The energy for dewatering was therefore calculated as:

$$E_{dewatering} = 1.0054 \times 1{,}334 m^3 = 1{,}341\ kWh \quad \text{(B.1-48)}$$

The centrifuges were assumed to recovery 90% of total solids, the mass of total solids was therefore calculated to be 29,753 kg. The total solids concentration of the

concentrate was assumed to be 250 kg/m^3, the flow of concentrate was therefore calculated as:

$$Q_{concentrate} = 29{,}753kg \times \frac{1}{250\frac{kg}{m^3}} = 119m^3 \quad \text{(B.1-49)}$$

The remaining flow that was send back to the primary clarifier was therefore calculated as:

$$Q_{remaining} = 1{,}334m^3 - 119m^3 = 1{,}215m^3 \quad \text{(B.1-50)}$$

B.3.10 Biogas production

The volume of biogas generated from sludge varies depending upon the proportions of carbohydrates, lipids and proteins in the sludge [254]. Biogas production values were used from a study [254] which presented values of 790, 1250 and 700 L biogas/kg VSS removed for carbohydrates, lipids and proteins respectively. The sludge characteristics were taken from the study by Levin [255] who found that primary sludge was composed of 20% carbohydrate, 27% lipid and 46% protein with 7% being an-organic material. For secondary sludge, the values were 42%, 10% and 48% for carbohydrates, lipids and proteins respectively. Combining the biogas production values and the sludge compositions, the biogas yields were calculated to be 0.818 m^3/ kg VSS for primary sludge and 0.793 m^3/kg VSS for secondary sludge.

The mass of VSS of both primary and secondary sludge into the reactor was 37.025 kg and 19,674 kg respectively considering 10% of the sludge is lost in the thickening process. Considering the volatile solids destruction rate of 50.4%, the total biogas production was calculated as:

$$V_{biogas} = 43{,}559kg \times 0.504 \times 0.818m^3 + 23{,}1460kg \times 0.504 \times 0.793m^3 = 23{,}148\ m^3 \quad \text{(B.1-51)}$$

According to the study by Rybicki [254] the methane content of biogas from primary sludge is 61.0% and 61.9% for secondary sludge. Given the sludge proportions, the methane content was therefore calculated to be 61.6%.

B.3.11 Co-generation of biogas

The produced biogas was assumed to be used in a co-generation turbine producing both electricity and heat. This process was modelled using data from Ecoinvent where it is reported that for every 1 MJ (lower heating value) of biogas fed to the turbine, 0.55 MJ of heat and 0.32 MJ of electricity are produced. The biogas was assumed to have a methane content of 63.3% with a lower heating value of 22.73 MJ/m^3. In this study the methane content was calculated as 61.6%, the lower heating value was therefore adjusted to 22.12 MJ/m^3.

For a biogas yield of 23,178 m^3, the produced values of electricity and heat were calculated as:

$$E_{electricity} = 23{,}148\, m^3 \times 22.12 \frac{MJ}{m^3} \times 0.32 \frac{MJ}{MJ} \times \frac{1}{3.6} \frac{kWh}{MJ} = 45{,}514.2\, kWh \quad \text{(B.1-52)}$$

$$E_{heat} = 23{,}148\, m^3 \times 22.12 \frac{MJ}{m^3} \times 0.55 \frac{MJ}{MJ} = 281{,}619\, MJ \quad \text{(B.1-53)}$$

As the WWTP currently operates biogas co-generation turbines, the input and related emissions of the co-generation components were not included in the study. The use and disposal of lubricating oil was included, according to Ecoinvent, for every 1 kWh produced, 2.6118 × 10^{-4} kg of lubricating oil is used and for 1 MJ of heat, 1.2334 × 10^{-5} kg is used. The total mass of lubricating oil was therefore calculated as:

$$M_{oil} = 45{,}515.2\, kWh \times 2.6118 \times 10^{-4} \frac{kg}{kWh} + 281{,}619\, MJ \times 1.2334 \times 10^{-4} \frac{kg}{MJ} = 15.4\, kg \quad \text{(B.1-54)}$$

B.4 Scenario 2

Scenario 2 assumes a similar set up to the existing wastewater treatment works, treating a higher volume of wastewater with the addition of algal raceway ponds following the secondary clarification for improved nutrient removal.

B.4.1 Primary clarification

The inflow to the primary clarifier including the returned flow from latter processes (sludge thickening and centrifugation) was calculated as 125,630 m^3. Using the equation from S1, the electricity consumption of the sludge scraper was calculated as:

$$E_{scraper} = 0.0039 \times 125{,}630 + 16.72 = 507\ kWh \quad \text{(B.1-55)}$$

For a calculated total suspended solids concentration of 0.713 kg/m^3 entering the primary clarifier, the mass of sludge scraped from the clarifier assuming a 55% removal rate was calculated as:

$$M_{primary\ sludge} = 0.55 \times 0.713\,\frac{kg}{m^3} \times 125{,}630\ m^3 = 49{,}260\ kg \quad \text{(B.1-56)}$$

Assuming a total solids density of 37 kg/m^3, the flow of sludge was calculated as:

$$Q_{primary\ sludge} = 49{,}260\ kg \times \frac{1}{37\ kg/m^3} = 1{,}305\ m^3 \quad \text{(B.1-57)}$$

B.4.2 Aeration tank

The aeration tank was based on the tank that is currently in use at Haifa WWTP. The tank uses low speed aerators to provide the oxygen necessary for bacterial respiration. The Influent concentrations of BOD and NH_3 to the aeration tank were 333 mg/L and 75 mg/L respectively, the concentrations considered the influent concentrations to the WWTP, the addition of recycled wastewater and the removal from primary clarification.

The mass of oxygen required for the BOD and NH_3 removal was calculated using the following equation from Metcalf and Eddy [200]:

$$R_o = Q(S_0 - S) - 1.42P_{x,bio} + 4.33Q(NO_x) \quad \text{(B.1-58)}$$

Where:

Q = influent flow

S_0 = influent bCOD concentration

S = effluent bCOD concentration

$P_{x,bio}$ = Weight of biomass, derived from degradable VSS

NO_x = Nitrified ammonia concentration

The mass of biomass required for the reduction of these concentrations to the levels reported in the WWTP report after the aeration process (10 mg BOD/L and 52 mg NH_3/L) was calculated using the equation from Metcalf and Eddy [200] for sludge production. The values of constants are: Y=0.4 g VSS/g bCOD, F_d=0.15, k_d=0.12 L/d, SRT=1.5 d, k_{dn}=0.08. The mass of biomass was calculated as:

$$P_{x,bio} = \frac{124{,}325\ m^3 \times 0.4 \times (0.531 - 0.016)\frac{kg}{m^3} \times (1 + 0.15 \times 0.12 \times 1.5)}{1 + 0.12 \times 1.5} + \frac{124{,}325\ m^3 \times 0.12 \times 0.0117\frac{kg}{m^3}}{1 + 0.08 \times 1.5} = 22{,}426\ kg \quad \text{(B.1-59)}$$

The mass of oxygen required was calculated as:

$$R_o = 124{,}325 \times (0.531 - 0.016) - 1.42 \times 22{,}426 + 4.33 \times 125{,}131 \times (0.064 - 0.052) = 38{,}416\ kg \quad \text{(B.1-60)}$$

The standard oxygen transfer rate was obtained from Metcalf and Eddy [200] using the average of the range of values presented for surface low-speed aerators, 1.8 kg O_2/kWh.

The transfer rate under field conditions was calculated using a relationship determined by Metcalf and Eddy [200] based on the standard oxygen transfer rate and field conditions. The field transfer rate was calculated as:

$$N_0 = 1.8\frac{kgO_2}{kWh} \times \frac{(0.95 \times 10.83) - 2}{9.17}\frac{mg}{L} \times 1.024^{20-20} \times 0.82 = 1.34\ \frac{kgO_2}{kWh} \quad \text{(B.1-61)}$$

The oxygen saturation concentration (10.83 mg/L) was calculated for a tank mid-depth of 2 m at 20°C.

For the oxygen consumption rate calculated, the energy consumption of the aerators was calculated as:

$$E_{aerator} = \frac{38{,}416\,\frac{kgO_2}{day}}{1.36\frac{kgO_2}{kWh}} = 28{,}769\ kWh \qquad \text{(B.1-62)}$$

B.4.3 Secondary clarification

The inflow to the secondary clarifier was calculated to be 124,325 m^3. The energy consumption was calculated using the method used in scenario 1 and was calculated as:

$$E_{scraper} = 0.0039 \times 124{,}325 + 16.72 = 502\ kWh \qquad \text{(B.1-63)}$$

As was carried out in S1, the mass of sludge obtained from the secondary clarification was calculated based on the equation for solids production during biological treatment in Metcalf and Eddy [200]. The variable values used are as follows: Y=0.4 g VSS/gbCOD, k_d=0.12 L/d, f_d=0.15, SRT=1.5 d. The initial BOD concentration was calculated to be 0.33 kg/m^3, the final BOD concentration was 0.01 kg/m^3. The initial NH_4 concentration was calculated to be 0.061 kg/m^3 and the concentration following aeration was 0.052 kg/m^3 as reported by the WWTP [205]. The initial TSS concentration was 0.324 kg/m^3 and assuming 10% of volatile suspended solids was non-biodegradable, the bVSS concentration was calculated to be 0.248 kg/m^3. The sludge mass was calculated as:

$$M_{sludge} =$$

$$124{,}325 m^3 \times \left(0.4\,\frac{gVSS}{gbCOD} \times 1.6 \times \left(0.330\,\frac{kg}{m^3} - 0.010\,\frac{kg}{m^3}\right) \times \frac{1+0.12\frac{L}{d}\times 0.15\times 1.5\ d}{0.85\times\left(1+0.12\frac{L}{d}\times 1.5\ d\right)} + \left(0.062\,\frac{kg}{m^3} - 0.052\,\frac{kg}{m^3}\right) \times \frac{0.12\frac{L}{d}}{1+0.08\times 1.5 d} + \left(0.324\,\frac{kg}{m^3} - 0.248\,\frac{kg}{m^3}\right)\right) = 35{,}690\ kg \qquad \text{(B.1-64)}$$

The solids content of the secondary sludge was assumed to be 0.8%. The flow sent to the anaerobic digestion facility was therefore calculated as:

$$Q_{secondary\ sludge} = 35{,}690\ kg \times \frac{1}{0.008\times 1000\ kg/m^3} = 4{,}461\ m^3 \qquad \text{(B.1-65)}$$

B.4.4 Gravity belt thickening

The sludge thickener was calculated using the same relationship as S1. The inflow of sludge to the thickener was calculated as 5,766 m^3, the energy consumption was therefore calculated as:

$$E_{GBT} = (0.0004 \times 5{,}766 + 0.825) \times 24 = 75\ kWh \quad \text{(B.1-66)}$$

As with S1, it was assumed that the gravity belt thickening process recovered 90% of the total solids and produced a concentrate with a total solids concentration of 5%. The mass of sludge recovered from the thickener was therefore calculated as:

$$M_{sludge} = 0.9 \times 84{,}950kg = 76{,}455kg \quad \text{(B.1-67)}$$

The corresponding flow was based on a density of 1000 kg/m^3 and was calculated as:

$$Q_{sludge} = 76{,}455kg \times \frac{1}{1000\frac{kg}{m^3} \times 0.05} = 1{,}529m^3 \quad \text{(B.1-68)}$$

The flow of effluent returned to the primary clarifier was calculated as:

$$Q_{return} = 5{,}766m^3 - 1{,}529m^3 = 4{,}237m^3 \quad \text{(B.1-69)}$$

B.4.5 Anaerobic digestion

The inputs to the anaerobic digestion were calculated using the same method as S1.

The electricity consumption for mixing was calculated as:

$$E_{mixing} = 1{,}529m^3 \times 10d \times 0.0065\ kw/m^3 \times 24h = 2{,}385\ kWh \quad \text{(B.1-70)}$$

The energy required to heat the sludge was calculated as:

$$E_{heat} = 4.18\frac{MJ}{t℃} \times 1{,}529\ t \times (35 - 20)℃ = 95{,}875\ MJ \quad \text{(B.1-71)}$$

The heat lost through the walls, roof and floor of the digester was assumed to be 10% of the total heat required to heat the sludge as the environmental temperature in Israel is similar to the mesophilic temperature. The extra heat required to account for the heat loss was therefore calculated as 9,588 MJ. The existing infrastructure was

assumed to be sufficient for the anaerobic digestion and therefore no extra materials were necessary.

The same percentage of volatile solids removal was calculated for S2 as S1 because the sludge retention time was the same, the removal rate was 50.44%.

The mass of sludge released from the anaerobic digester was therefore calculated as:

$$M_{sludge} = 76{,}455\ kg \times (1 - 0.5044) = 37{,}891 kg \quad \text{(B.1-72)}$$

B.4.6 Sludge dewatering

As with S1, sludge dewatering was conducted using centrifuges. A sludge flow of 1,529 m^3 was calculated as entering the centrifuges. The electricity consumption was calculated using the same equation as scenario 1:

$$E_{dewatering} = 1.0054 \times 1{,}529 m^3 = 1{,}537\ kWh \quad \text{(B.1-73)}$$

B.4.7 Biogas production

The volume of biogas generated from the sludge was calculated using the same method used for scenario 1. The mass of VSS of both primary and secondary sludge into the reactor was 37,684 kg and 27,303 kg respectively considering 10% of the sludge is lost in the thickening process. The volatile solids destruction was calculated as 50.4%. The total biogas production was calculated as:

$$V_{biogas} = (37{,}684 kg \times 0.818 m^3 + 27{,}303 kg \times 0.793 m^3) \times 0.504 = 26{,}472\ m^3 \quad \text{(B.1-74)}$$

According to the study by Rybicki [254] the methane content of biogas from primary sludge is 61.0% and 61.9% for secondary sludge. Given the sludge proportions, the methane content was therefore calculated to be 61.6%.

B.4.8 Water pumped to algae ponds

A total pond area of 308.6 ha was calculated to be necessary for full treatment of the wastewater (see below). The pond array dimensions were a length of 3,086 m and a width of 1,000 m. One large pipe was assumed to transport the effluent through the centre of the array where smaller pipes transported the effluent to the ponds. The mean

length of distance travelled for the effluent in the main pipe was therefore 1,543 m. The flow of effluent was calculated to be 1.387 m^3/s. The energy consumption depended upon the total dynamic head of the pipes. The static head was 0.3 m as a result of the pond depths. The frictional head losses were calculated based on typical equations in hydraulic engineering. The diameter of the primary pipe was assumed to be 1 m, the velocity of the effluent was therefore calculated as 1.77 m/s, the kinematic viscosity value was 1.13×10^{-6} m^2/s. Using these values, the Reynolds number was calculated as:

$$R_e = \frac{1m \times 1.77\frac{m}{s}}{1.13 \times 10^{-6}\frac{m^2}{s}} = 1.56 \times 10^6 \quad \text{(B.1-75)}$$

To calculate the frictional head using the Darcy–Weisbach formula it was necessary to calculate the value of λ using the moody formula (the k_s value for plastic of 0.03 mm was used):

$$\lambda = 0.0055\left(1 + \left(\frac{20{,}000 \times 3 \times 10^{-5}}{1m} + \frac{10^6}{1.56 \times 10^6}\right)^{\frac{1}{3}} = 0.016 \quad \text{(B.1-76)}$$

Using the Darcy-Weisbach formula the frictional head loss was calculated as:

$$h_f = \frac{0.016 \times 1{,}543m \times 1.77^2\left(\frac{m}{s}\right)^2}{2 \times 9.81\frac{m}{s^2} \times 1\ m} = 3.93\ m \quad \text{(B.1-77)}$$

The total dynamic head included the local head losses where the water entered the secondary pipes at a 90° angles. The water was assumed to enter 40 secondary pipes, the k_L value of the joints was taken as 0.5. The value of h_L was calculated as:

$$h_L = \frac{40 \times 0.5 \times 1.77^2(\frac{m}{s})^2}{2 \times 9.81\frac{m}{s}} = 3.18\ m \quad \text{(B.1-78)}$$

The frictional head values of the secondary pipes were calculated using the same method for a 0.5 m diameter and a flow of 0.035 m^3/s. The calculated value of frictional head was 1.06 m, as each pipe was assumed to transport effluent to 10 ponds, 400 T-junctions of 90° were assumed requiring an additional head loss of 0.32 m

The total dynamic head was calculated to be 8.8 m. Considering a pump efficiency of 80%, the electricity consumption was therefore calculated as:

$$E_{pump} = \frac{119{,}864}{60\times60}\frac{m^3}{s} \times 9.81\,\frac{g}{m^2} \times 1000\,\frac{kg}{m^3} \times 8.8\,m \times \frac{1}{0.8} \times \frac{1}{1000}\frac{kW}{W} = 4{,}784\,kWh \quad \text{(B.1-79)}$$

B.4.9 Nutrient uptake in ponds

The uptake of nutrients was calculated based on the productivity of the algal biomass and the content of nitrogen and phosphorous in the biomass. The growth rate was based on research conducted previously at the Technion Israel Institute of Technology by Professor Shelef who investigated growth of algae in raw wastewater [47]. Professor Shelef observed that native strains of algae tended to dominate and the strains varied between seasons. The productivity also varied greatly between the seasons, the maximum productivity being 50 g/m^2/day in July reducing to 0.4 g/m^2/day in December. Obviously a productivity as low as 0.4 g/m^2/day would not provide adequate removal of nutrients, therefore in the winter months an alternative method of nutrient recovery would be necessary. For this study, an average productivity of 25.6 g/m^2/day was assumed. Values similar to this have been used in various other studies [33, 35]. The general molecular formula for green algae is $C_{106}H_{263}O_{110}N_{16}P$ [256] was used. Therefore, considering stoichiometry, nitrogen makes up 0.0631 g/g of the biomass and phosphorous 0.00873 g/g. One gram of cultivated biomass was therefore assumed to take up 0.0766 g of NH_3. As the depth was considered to be 0.3 m and the productivity, 25.6 g/m^2/day, the uptake of NH_3 was calculated as:

$$NH_3 uptake = 76.6\,\frac{mg}{g} \times 25.6\,\frac{g}{m^2.d} \times \frac{1}{300}\frac{m^2}{L} = 6.54\,\frac{mg}{L.d} \quad \text{(B.1-80)}$$

The uptake of P was calculated as:

$$P_{uptake} = 8.73\,\frac{mg}{g} \times 25.6\,\frac{g}{m^2.d} \times \frac{1}{300}\frac{m^2}{L} = 0.75\,\frac{mg}{L.d} \quad \text{(B.1-81)}$$

The concentration of NH_3 in the influent water was taken as 52 mg/L and 4.9 mg/L of TP [205]. The number of days to reduce these nutrient levels to 1.5 mg/L and 1 mg/L for NH_3 and P considering the uptake rate was calculated to be 7.7 days for NH_3 and

6.6 days for P. Therefore the assumed hydraulic retention time was 7.7 days. A supply of P would potentially be necessary to continue the productivity of the algal biomass once P became limited. The supply of P from the wastewater was assumed to last 6.6 days, for the remaining 1.1 day superphosphate was assumed to be added. For continued growth, the mass of phosphorous required was calculated as:

$$M_P = 8.73\frac{mg}{g} \times 25.6\frac{g}{m^2.d} \times 1.1d \times 3.09 \times 10^6 m^2 \times \frac{1}{1\times10^6}\frac{kg}{mg} = 792\ kg \quad \text{(B.1-82)}$$

The additional phosphorous was considered to be added as superphosphate, the impacts of which are determined as the mass of P_2O_5 by Ecoinvent [106], the equivalent mass of P_2O_5 was calculated to be 1,812 kg based on stoichiometry.

For a hydraulic retention time of 7.7 days and a depth of 0.3 m, the total pond area necessary was calculated as:

$$A_{pond} = 119{,}863\ m^3 \times 7.7\ d \times \frac{1}{0.3\ m} = 3{,}086{,}016\ m^2 \quad \text{(B.1-83)}$$

B.4.10 Paddlewheel electricity consumption

The electricity consumption of the paddle was based on the study by Clarens *et al.* [27] where it was estimated that the power rating of each paddlewheel is 0.037 kW and each wheel services 100 m^2 of pond area. The energy consumption was therefore calculated as:

$$E_{paddlewheel} = \frac{3{,}086{,}016m^2}{100m^2} \times 0.037\ kW \times 24\ h = 27{,}404\ kWh \quad \text{(B.1-84)}$$

B.4.11 Concrete infrastructure

All of the pond infrastructure was assumed to be produced from concrete with a block wall width of 0.1 m and depth of 0.4 m. The blocks are assumed to be on top of a 0.1 m thick concrete slab.

The total block volume for one pond was calculated as:

$$V_{block} = (2 \times 100.2m \times 0.1m \times 0.4m) + (2 \times 10m \times 0.1m \times 0.4m) = 8.82m^3 \quad \text{(B.1-85)}$$

The total number of ponds was calculated to be 3,086, the density of concrete was assumed to be 2,380 kg/m^3 and the life span of the ponds was assumed to be 20 years. The concrete block input was calculated as:

$$M_{block} = 8.82m^3 \times 3{,}086\ ponds \times 2{,}380\frac{kg}{m^3} \times \frac{1}{365\ days \times 20\ years} = 8{,}870\ kg \quad \text{(B.1-86)}$$

The volume of the concrete base was calculated as:

$$V_{base} = 3{,}086{,}016\ m^2 \times 0.1m^3 \times \frac{1}{365\ days \times 20\ years} = 42.3m^3 \quad \text{(B.1-87)}$$

B.4.12 Flue gas injection

The flue gas from the co-generation turbine was assumed to be pumped into the ponds to use the CO_2 and avoid environmental emissions. The energy requirement to deliver the gas from the turbine to the pond was based on the work by Kadam [48]. From this work, a value of 22 kWh/t CO_2 was assumed. The mass of CO_2 emitted from the turbine was calculated based on the emission values of biogenic CO_2 reported by Ecoinvent [106] or the production of 1 kWh of electricity (0.727 kg CO_2) and 1 MJ of heat (0.0343 MJ) for a biogas co-generation engine. The amount of energy generated varied for each process stream, the CO_2 emission values were calculated as 73,533 kg, 73,000 kg, 79,301 kg and 68,429 kg for S2 A, B, C and D respectively. The energy consumption to inject this gas for S2 A was calculated as:

$$E_{fluegas} = 22\frac{kWh}{tCO_2} \times 73.5\ t = 1{,}632\ kWh \quad \text{(B.1-88)}$$

For S2 B, C and D, the energy consumption values were 1620 kWh, 1,760 kWh and 1,519 kWh respectively.

B.4.13 Flocculation tank

The volume of water pumped into the flocculation tank was lower than the water into the ponds due to evaporation which was taken to be an average of 2.2 mm once rainfall had been subtracted [257]. The total daily flow was 67, 335 m^3. A similar network of pipes was assumed for transporting the effluent from the ponds as used for transporting the effluent from the WWTP to the ponds. The secondary pipes were

assumed to have a diameter of 0.3 m corresponding to a velocity of 0.28 m/s. The kinematic viscosity was assumed to be the same as water, 1.13×10^{-6} m^2/s. The Reynold's number was calculated as:

$$R_e = \frac{0.3\ m \times 0.28 \frac{m}{s}}{1.13\times10^{-6} \frac{m^2}{s}} = 7.32 \times 10^4 \quad \text{(B.1-89)}$$

To calculate the frictional head using the Darcy –Weisbach formula it was necessary to calculate the value of λ using the moody formula (the k_s value for plastic of 0.03 mm was used):

$$\lambda = 0.0055\left(1 + \left(\frac{20{,}000\times3\times10^{-5} m}{0.3\ m} + \frac{10^6}{7.32\times10^4}\right)^{\frac{1}{3}} = 0.034 \quad \text{(B.1-90)}$$

Using the Darcy-Weisbach formula the frictional head loss was calculated as:

$$h_f = \frac{0.034\times10{,}000\ m \times 0.28^2 \left(\frac{m}{s}\right)^2}{2\times9.81 \left(\frac{m}{s^3}\right)\times0.3m} = 4.38\ m \quad \text{(B.1-91)}$$

The total dynamic head included the local head losses where the water entered the secondary pipes at a 90° angles. The water was assumed to enter 40 secondary pipes, the k_L value of the joints was taken as 0.5. The value of h_L was calculated as:

$$h_L = \frac{40\times0.5\times0.28^2 \left(\frac{m}{s}\right)^2}{2\times9.81 \frac{m}{s^2}} = 0.08\ m \quad \text{(B.1-92)}$$

The secondary pipes were assumed to connect to a large main pipe with a diameter of 0.75 m and a corresponding velocity of 1.76 m/s. The Reynold's number was calculated as:

$$R_e = \frac{0.75\ m \times 1.76 \frac{m}{s}}{1.13\times10^{-6}\ m^2/s} = 1.17 \times 10^6 \quad \text{(B.1-93)}$$

Using the moody formula the value of λ was calculated as:

$$\lambda = 0.0055\left(1 + \left(\frac{20{,}000\times3\times10^{-5}\ m}{0.75\ m} + \frac{10^6}{1.17\times10^6}\right)^{\frac{1}{3}} = 0.017 \quad \text{(B.1-94)}$$

Using the Darcy-Weisbach formula the frictional head loss was calculated as:

$$h_f = \frac{0.017 \times 1{,}540\ m \times 1.76^2 \left(\frac{m}{s}\right)^2}{2 \times 9.81 \frac{m}{s^2} \times 0.75\ m} = 5.57\ m \quad \text{(B.1-95)}$$

The main pipe was assumed to use two 90 degree bends to transport the effluent to the flocculation tank , the k_L value of the joints was taken as 0.5. The value of h_L was calculated as:

$$h_L = \frac{2 \times 0.5 \times 1.76^2 \left(\frac{m}{s}\right)^2}{2 \times 9.81\ m/s^2} = 0.16\ m \quad \text{(B.1-96)}$$

The static head for the pump was 4 m, the assumed height of the flocculation tank. The total dynamic head was therefore calculated to be 14.2 m. Considering a pump efficiency of 80%, the electricity consumption was therefore calculated as:

$$E_{pump} = \frac{67{,}335}{60 \times 60} \frac{m^3}{s} \times 9.81 \frac{g}{m^2} \times 1000 \frac{kg}{m^3} \times 14.2\ m \times \frac{1}{0.8} \times \frac{1}{1000} \frac{kW}{W} = 3{,}255\ kWh \quad \text{(B.1-97)}$$

The energy consumption for mixing was calculated using the mechanical mixing formula from Metcalf and Eddy [200] where the value for G was 75/s, a typical value for flocculation of wastewater [200] and μ was 0.001 N.s/m^2 (water at 20°C). The tank volume was based on a retention time of 0.5 hours, a daily flow of 67,335 m^3 and a fill volume ratio of 0.7, the tank volume was calculated to be:

$$V_{tank} = 67{,}335\ m^3 \times \left(\frac{0.5h}{24h}\right) \times \frac{3}{2} = 2{,}004 m^3 \quad \text{(B.1-98)}$$

The energy consumption for mixing was therefore calculated as:

$$E_{mixing} = 2{,}004\ m^3 \times 1 \times 10^{-3} N. \frac{s}{m^2} \times \frac{75^2}{s^2} \times \frac{1}{1000} \frac{kW}{W} \times 24h = 271\ kWh \quad \text{(B.1-99)}$$

4 flocculation tanks where assumed to be used, the total volume for each was therefore 351 m^3. A fill volume of 0.7 was assumed, therefore the tank volumes were 501 m^3. Assuming a 4 m depth, the area for each tank was 125.3 m^2, the lengths were therefore 11.2 m. A wall width of 0.3 m was used, the volume of concrete block was therefore calculated as:

$$V_{block} = 4 \times (11.2m \times 2 + 11.8m \times 2) \times 4m \times 0.3m = 220.6\ m^3 \quad \text{(B.1-100)}$$

The flocculation tanks were assumed to last 30 years and the concrete was assumed to have a density of 2,380 kg/m^3. The input mass of concrete block was therefore calculated as:

$$M_{block} = 220.6m^3 \times 2{,}380\,\frac{kg}{m^3} \times \frac{1}{30\,y \times 365\,d} = 48\,kg \quad \text{(B.1-101)}$$

The base of each tank was assumed to be 0.3 m thick. The volume was therefore calculated as:

$$V_{base} = 11.8m \times 11.8m \times 0.3m = 166.8\,m^3 \quad \text{(B.1-102)}$$

Assuming a life span of 30 years, the input volume of concrete for the base was calculated to be 0.02 m^3.

B.4.14 Sedimentation tank

The sedimentation tank was assumed to have a depth of 3 m, the pump energy to transport the effluent from the flocculation tank to the sedimentation was calculated based only on this static head. The energy consumption was calculated as:

$$E_{pump} = \frac{67{,}335}{60 \times 60}\frac{m^3}{s} \times 9.81\,\frac{g}{m^2} \times 1000\,\frac{kg}{m^3} \times 3\,m \times \frac{1}{0.8} \times \frac{1}{1000}\frac{kW}{W} = 688\,kWh \quad \text{(B.1-103)}$$

The energy consumption of the sludge scraper was calculated using the same method as for the WWTP. The energy consumption was calculated as:

$$E_{scraper} = 0.0039 \times 67{,}335 + 16.72 = 279\,kWh \quad \text{(B.1-104)}$$

4 tanks were assumed to be used for sedimentation, the tanks were assumed to be circular with a depth of 3m and a wall width of 0.3m. The total required volume was calculated to be 1,403 m^3 based on a retention time of 0.5 hours. The area of each tank was therefore calculated to be 116.9m^2 with a radius of 6.1 m. The outside radius was therefore 6.4 m using a 0.3 m width. The total concrete volume was calculated as:

$$V_{block} = (\pi \times 6.4^2\,m^2 - 116.9\,m^2) \times 3m = 35.3\,m^3 \quad \text{(B.1-105)}$$

Assuming a life span of 30 years and a concrete density of 2,380 kg/m^3 the mass of concrete block was calculated as:

$$M_{block} = 4 \times 35.3\ m^3 \times 2{,}380 \frac{kg}{m^3} \times \frac{1}{365\ d \times 30\ y} = 30.7\ kg \quad \text{(B.1-106)}$$

The floor slab was assumed to have a depth of 0.3 m. The volume of slab was calculated as:

$$V_{slab} = 4 \times 0.3m \times \pi \times 6.4^2 m^2 = 154.4\ m^3 \quad \text{(B.1-107)}$$

Similarly to the blocks, the life span was assumed to be 30 years, the input was therefore 0.014 m^3 of normal concrete.

B.4.15 Flocculation materials

Flocculation was assumed to be conducted using chitosan which has been shown to be an effective flocculant for many types of algal biomass [53, 87, 88]. A loading of 15 mg/L was assumed which has been proven effective for good removal of algal biomass [88]. For a flow of 67,335 m^3 the required mass of flocculant was calculated to be:

$$M_{flocculant} = 0.015 \frac{kg}{m^3} \times 67{,}335 m^3 = 1{,}010\ kg \quad \text{(B.1-108)}$$

No data was available for the cumulative energy demand of producing chitosan or the environmental impacts, the impact was therefore neglected however this input should be noted.

B.4.16 Centrifugation

The algal sludge from the sedimentation tank was assumed to be pumped a height of 1 m to the centrifuge, the frictional head was considered negligible. The flow of sludge was calculated based on the algal sludge density measured by Mohn [82] who recovered 1.5% total solids following sedimentation. A 10% loss of algal biomass was assumed following sedimentation, the recovery of total solids was therefore calculated to be 71,102 kg. With a total solids content of 1.5% the flow was calculated to be 4,740m^3. The pump efficiency was assumed to be 0.6. The energy for pumping was therefore calculated as:

$$E_{pump} = \frac{4{,}740}{60 \times 60} \frac{m^3}{s} \times 9.81 \frac{g}{m^2} \times 1000 \frac{kg}{m^3} \times 1\ m \times \frac{1}{0.6} \times \frac{1}{1000} \frac{kW}{W} = 21.5\ kWh \quad \text{(B.1-109)}$$

The energy consumption of the centrifuge was assumed to be 1 kWh/m^3 which has been reported as the consumption for the Westfalia self-cleaning, disk-stack centrifuge by Molina Grima *et al.* [145] and also for the Alpha Laval OFX40 nozzle centrifuge [145]. The energy consumption of the centrifuge was therefore calculated as:

$$E_{centrifuge} = 4{,}740 m^3 \times 1 \frac{kWh}{m^3} = 4{,}740\ kWh \quad \text{(B.1-110)}$$

Each centrifuge was assumed to have a maximum capacity of 43 m^3/h which is the maximum nozzle capacity published by Alfa Laval for the OXF40 centrifuge [258]. The number of centrifuges required was therefore calculated as:

$$N_{centrifuge} = \frac{4{,}740 m^3}{43 \frac{m^3}{h} \times 24\ h} = 4.59 \approx 5 \quad \text{(B.1-111)}$$

According to the Alfa Laval specifications, the total weight of each centrifuge is 12,700 kg, therefore the total weight is 63,500 kg. The centrifuges were assumed to have a life span of 20 years and the mass was assumed to be chrome steel. The input mass was calculated as:

$$M_{centrifuge} = 63{,}500\ kg \times \frac{1}{20\ y \times 365\ d} = 8.70\ kg \quad \text{(B.1-112)}$$

B.4.17 Homogenization

Homogenization of the biomass was assumed to be conducted with a wet biomass attritor modelled on the Q-100 Union Process wet biomass circulation attritor [259]. The pumping rate of the attritor is gal/min (591 L/min), the average power rating is 125 h (93.2 kW) and the mass is 9,900 lb (902.7 kg). One unit was calculated to provide a sufficient throughput for the system. The time to grind all of the biomass was calculated as:

$$T_{grinding} = \frac{675{,}467 L}{591 \frac{L}{\text{min}}} \times \frac{1}{60} h/\text{min} = 19\ h \quad \text{(B.1-113)}$$

The energy consumption was therefore calculated to be:

$$E_{grinding} = 93.2\ kW \times 19h = 1{,}776\ kWh \quad \text{(B.1-114)}$$

The attritor was assumed to be produced from chromium steel and the life span was assumed to be 10 years, the input mass was therefore calculated as:

$$M_{grinding} = 902.7\ kg \times \frac{1}{10\ y \times 365\ d} = 0.25 kg \quad \text{(B.1-115)}$$

B.4.18 Bioenergy production

Four bioenergy production scenarios were considered in this study, there were:

A – Biodiesel and biogas production

B – Bioethanol and biogas production

C – Biogas production

D – Biodiesel, bioethanol and biogas production

The inputs to the main processes are first considered in detail followed by the inputs for the combined processes.

B.4.18.1 Biodiesel production

Where the process stream included the production of biodiesel, two processes were modelled: lipid extraction and trans-esterification. Both processes were modelled using data from Ecoinvent v2.2 [106] for the production of biodiesel from soy beans.

Lipid extraction

The data for lipid extraction from soy beans published by Ecoinvent is determined for 1 kg of soy bean [219]. The moisture content of algal biomass is different to soy beans, the inputs were therefore adjusted to 1 kg of dry solids. The moisture content of soy beans used by Ecoinvent was 11%. Table 2 displays the input data for 1kg of soy bean (w.w) and 1 kg of soy bean dry matter. Ecoinvent allocates the inputs between the oil produced and the soy meal [219], this study assumed all of the inputs are for oil extraction. Ecoinvent assumes heat is produced from various sources, this study assumes heat is provided by the same source. Ecoinvent also assumes all hexane is recycled, this study assumes that 10% of the input hexane is lost in the process. The

total mass of solids following centrifugation was calculated to be 67, 547 kg. Table B-3 displays the input values calculated for the total mass of dry solids. Table B-4 displays the inputs for the total mass of algal biomass into the oil mill.

Table B-3 Ecoinvent inputs for the oil extraction of 1 kg of soy bean (w.w) and 1 kg soy bean (d.w)

Input	1 kg soy bean (w.w)	1 kg soy bean (d.w)
Electricity	0.0562 kWh	0.0631 kWh
Heat	0.978 MJ	1.099 MJ
Hexane	2.14×10^{-4} kg	2.40×10^{-4} kg
Phosphoric acid	1.69×10^{-3} kg	1.90×10^{-4} kg
Tap water	0.157 kg	0.176 kg
Wastewater treatment	1.69×10^{-4} m^3	1.90×10^{-4} m^3
Oil mill	3.13×10^{-10}	3.52×10^{-10}

Table B-4 Inputs to the model for the oil extraction of the total algal biomass

Input	Total input value
Electricity	4,265 kWh
Heat	74,225 MJ
Hexane	16.2 kg
Phosphoric acid	12.8 kg
Tap water	11,916 kg
Wastewater treatment	12.8 m^3
Oil mill	2.38×10^{-5}

Transesterification

The inputs for the transesterification of the extracted oil were calculated based on the mass of methyl-esthers produced. The biomass was assumed to contain 20% oil and the extraction process was considered to be 95% efficient [260], the mass of produced oil was therefore calculated to be 12,834 kg. The transesterification process was assumed to be 96% efficient [91], therefore 12,321 kg of methyl esters were calculated as being produced. Table B-5 displays the inputs for 1 kg of methyl ester as well as the inputs for the total mass of methyl esters produced. Ecoinvent allocates a proportion of the inputs to glycerine as a by-product but no allocation was used in this study for the transesterification process.

Table B-5 Input values for 1kg of produced methyl esters and the total model input

Input	1 kg methyl esters	Total input value
Electricity	0.0761 kWh	937.6 kWh
Heat	0.850 MJ	10,476 MJ
Hydrochloric acid (30%)	4.236×10^{-3}	52.2 kg
Methanol	0.105 kg	1,288 kg
Phosphoric acid	1.05×10^{-2}	128.8 kg
Tap water	0.0252	309.9 kg
Wastewater	5.76×10^{-5} m^3	0.71 m^3
Vegetable oil esterification plant	8.60×10^{-10}	1.06×10^{-5}

Fermentation and distillation

The process of fermentation and distillation was based on the fermentation of corn using data from Ecoinvent. The data included the pre-treatment, saccharification, fermentation and distillation of the biomass. The energy input for stillage treatment was not included as the residual biomass was assumed to be used for anaerobic digestion. Similarly to the lipid extraction, the inputs for the fermentation and distillation were based on the dry mass of biomass being fermented. Table B-6 displays the inputs required for 1 kg of corn and the inputs for 1 kg of dry mass of corn. Table B-7 displays the inputs for the total mass of dry solids.

Table B-6 Ecoinvent inputs for the fermentation and distillation of 1 kg corn (w.w) and 1 kg corn (d.w)

Input	1 kg corn (w.w)	1 kg corn (d.w)
Electricity	0.1546 kWh	0.1798 kWh
Heat	3.366 MJ	3.914 MJ
Ammonium sulphate	0.003 kg	0.0035 kg
Diammonium phosphate	0.003 kg	0.0035 kg
Soda powder	0.0112 kg	00130 kg
Sulphuric acid	0.0075 kg	0.00872 kg
Ethanol fermentation plant	1.54×10^{-10}	1.79×10^{-10}

Table B-7 Total inputs for the fermentation and distillation process for the model

Input	Total input value
Electricity	12,143 kWh
Heat	264,375MJ
Ammonium sulphate	236 kg
Diammonium phosphate	236 kg
Soda powder	880 kg
Sulphuric acid	589 kg
Ethanol fermentation plant	1.21×10^{-5}

Following distillation the bioethanol requires upgrading to 99.7% ethanol. The inputs necessary to upgrade the bioethanol are also based on data from Ecoinvent and are displayed in table B-8 for 1 kg of bioethanol, the total inputs are also displayed in the table. The yield of bioethanol from algae depends greatly upon many different factors, including species, pre-treatment methods, hydrolysis and fermentation conditions. Yields as high as 38% have been recorded [100] however the conversion rate used for this study was 10.3% which a rate obtained from a study that hydrolysed and fermented wild species of algae [261]. The total mass of bioethanol produced was calculated as:

$$M_{EtOH} = 67{,}547\ kg \times 0.103\ \frac{kg\ ethanol}{kg\ biomass} = 6{,}957\ kg \qquad \text{(B.1-116)}$$

Table B-8 Energy and plant inputs for 1kg bioethanol according to Ecoinvent and the total input for the model

Input	1 kg bioethanol	Total input value
Electricity	8.839×10^{-3} kWh	61.5 kWh
Heat	1.005 MJ	6,989 MJ
Ethanol fermentation plant	5.299×10^{-11}	3.69×10^{-7}

Anaerobic digestion

The anaerobic digestion was assumed to occur in a separate facility from the digestion of the wastewater sludge although co-digestion of biomass may be possible. The anaerobic digestion process was modelled using relationships determined for the anaerobic digestion of sewage sludge. A simple, concrete tank was assumed to be used

as the digestion tank with a mechanical mixer to mix the biomass. According to Metcalf and Eddy [200] the power requirement of the mixer is dependent upon the digester volume, the average value of the range published is 0.0065 kW/m^3 of digester volume. The digester volume was calculated based on the volume of biomass entering the digester and the retention time. The inflow to the digester was calculated to be 675.5 m^3 daily, a SRT of 30 days was assumed sufficient for biogas production, the digester volume was therefore calculated to be 20,264 m^3. For this volume the energy consumption for mixing was calculated as:

$$E_{mixing} = 0.0065 \frac{kW}{m^3} \times 20{,}264 m^3 = 3{,}161\ kWh \quad \text{(B.1-117)}$$

The digester heating was calculated considering the energy required to heat the biomass to the digester temperature and the heat loss of the digester. The heat capacity of the biomass was assumed to be the same as water, 4.18 kJ/kg.°C. The heat requirement was therefore calculated as:

$$E_{heat} = 4.18 \frac{kJ}{kg.°C} \times 675{,}467 kg \times (35 - 20)°C \times \frac{1}{1000} \frac{MJ}{kJ} = 42{,}352\ MJ \quad \text{(B.1-118)}$$

The heat loss in the reactor was assumed to be 10%, the extra heat required was therefore calculated to be 4,235 MJ. The walls of the digester were assumed to be built from 0.3 m thick concrete blocks with a depth of 23.9 m. The floor was assumed to be a conical slab with a mid-depth of 9.6 m. The roof was assumed to be a 100 mm thick, fixed concrete cover. The diameter of the tank was considered to be 30 m, the circumference of the tank was therefore 94.2 m. The volume of concrete block required for the wall was calculated as:

$$V_{wall} = 94.2m \times 23.9m \times 0.3m = 675.5m^3 \quad \text{(B.1-119)}$$

The density of the concrete was assumed to be 2,380 kg/m^3 and the life span of the tank was assumed to be 30 years. The input mass of concrete block was therefore calculated as:

$$M_{wall} = 675.5m^3 \times 2{,}380 \frac{kg}{m^3} \times \frac{1}{365d \times 30y} = 146.8kg \quad \text{(B.1-120)}$$

The volume of the floor slab was calculated as the area of the cone multiplied by the depth:

$$V_{slab} = \pi \times 9.6m \times 15m \times 0.3m = 251.4m^3 \quad \text{(B.1-121)}$$

The volume of the roof was calculated as:

$$V_{roof} = \pi \times 15^2 \times 0.1m = 70.7m^3 \quad \text{(B.1-122)}$$

Assuming a life span of 30 years, the input volume of concrete for the slab and the roof was therefore calculated as:

$$V_{AD} = 322.1m^3 \times \frac{1}{365d \times 30y} = 0.029m^3 \quad \text{(B.1-123)}$$

Biogas production

The methane yield used was obtained from the study by Golueke *et al.* [17] where algal sludge taken from a wastewater lagoon was digested at temperatures between 35 and 50°C. Data from this research was used because the biomass tested was a mix of species, which is most likely to occur in an algal pond. For mesophilic conditions (35°C), the methane yield of the algal sludge varied from 0.232 to 0.268 L CH_4/g VS and the CH_4 content varied from 60.9 to 62.4. An average yield of 0.250 L CH_4/g VS was used in this study. The values from the study are similar to those from more recent studies, Sialve *et al.* [36] reviewed methane recoveries from different studies reporting yields from 0.09 to 0.45 L CH_4/ g VS. In most cases the species were specifically selected. Assuming an average volatile solids content of 60%, the methane yield was calculated as:

$$V_{CH4} = 67{,}547\ kg \times 0.25 \frac{m^3\ CH_4}{kg\ VS} = 10{,}132\ m^3 \quad \text{(B.1-124)}$$

Using an average CH_4 content of 61.5%, this corresponded to a biogas volume of 16,476 m^3.

Biogas co-generation

Similarly to the biogas produced from the wastewater sludge, the co-generation of the biogas was modelled on data from Ecoinvent [106]. The biogas in the Ecoinvent report was assumed to have a methane content of 63.34% and a lower heating value of 22.73 MJ/m^3. With a CH_4 content of 61.5%, the biogas produced from the algal biomass was calculated to have a lower heating value of 22.07 MJ/m^3. The electricity and heat production values were calculated as:

$$E_{electricity} = 16{,}476\ m^3 \times 22.07 \frac{MJ}{m^3} \times 0.32 \times \frac{1}{3.6} \frac{kWh}{MJ} = 32{,}319\ kWh \quad \text{(B.1-125)}$$

$$E_{heat} = 6{,}476\ m^3 \times 22.07 \frac{MJ}{m^3} \times 0.55 = 199{,}977\ MJ \quad \text{(B.1-126)}$$

Co-generation components were assumed a necessary input for conversion of the biogas produced to electricity and heat. According to Ecoinvent, the components for electricity generation are 4.353×10^{-8} 160 kWe co-gen unit (components for heat and electricity) and 5.625×10^{-8} 160 kWe co-gen unit (components for electricity) per 1 kWh of electricity produced. For heat generation the values are 2.056×10^{-9} 160 kWe co-gen unit (components for heat and electricity) and 9.091×10^{-9} 160 kWe co-gen unit (components for heat) per 1 MJ of heat produced. 2.612×10^{-4} kg of lubricating oil was assumed per 1 kWh of electricity and 1.233×10^{-5} kg per 1 MJ of heat. The total inputs are displayed in table B-9.

Table B-9 Infrastructure requirements for biogas co-generation

Input	Value
160 kWe co-gen unit (components for heat and electricity)	0.00182
160 kWe co-gen unit (components for electricity)	0.00182
160 kWe co-gen unit (components for heat)	0.00182
Lubricating oil	10.9 kg

Combined processes

Each process stream apart from stream C combined processes to recover the maximum amount of energy. Anaerobic digestion was used in each stream, for process streams A, B and D it was used to reduce solid waste and recover more energy in the form of biogas. Process stream D combined all of the processes, following lipid extraction, the residual biomass was fermented and distilled with the remaining biomass then be digested. Different methods of processing the biomass are likely to impact the yields obtained from the biomass, in the case of fermentation following lipid extraction the yields were assumed to remain the same because different fraction of the biomass are used. The methane yields obtained from biomass are affected by the proportion of components, generally grouped into lipids, proteins and carbohydrates [36]. Lipids provide the best substrate for methane production, therefore following lipid extraction the methane yield is likely to be reduced. To account for this in the study, following lipid extraction (process streams A and D), the minimum biogas yield (0.238 m^3 CH_4/kg VS) was used for calculating the methane yield obtained from the residual mass. The bioethanol yield was not considered to be affected by lipid extraction.

S2 A Biodiesel and biogas production

Following the production of biodiesel, the residual biomass was assumed to be anaerobically digested to produce biogas. The calculations were the same for the production of only biogas, however because the influent biomass was less (54,713 kg), each of the inputs were lower. Table B-10 displays the inputs for the anaerobic digestion process for this scenario.

Table B-10 Inputs for anaerobic digestion following biodiesel production

Input	Value
Mixing	2,114 kWh
Heating	37,735 MJ
Concrete block	118.9 kg
Concrete, normal (slab/roof)	0.028 m^3

For a residual biomass input of 54,713 kg and a methane conversion of 0.23 L CH_4/g VS, the methane yield was calculated to be 8,207 m^3 or 13,345 m^3 biogas. This biogas yield corresponded to an electricity and heat production of 26,179 kWh and 161,981 MJ respectively.

To convert the biogas to electricity and heat, 0.00147 of a 160 kWe co-gen unit with components for electricity and heat, electricity only and heat only were required. The consumption and disposal of 8.8 kg of lubricating oil was also required.

S2 B Bioethanol and biogas production

The mass of biomass entering the anaerobic digester following fermentation was calculated based on the mass of bioethanol produced and the CO_2 released. The mass of CO_2 released was based on the relationship reported by Ecoinvent [219] where 1.015 kg of CO_2 is emitted for every 1 kg of anhydrous bioethanol produced. Therefore, for a biomass input of 67,547 kg and an ethanol recovery of 10.3% the biomass mass entering the anaerobic digester was calculated as:

$$M_{AD} = 67{,}547\ kg - 67{,}547\ kg \times 0.103 - 67{,}547\ kg \times 0.103 \times 1.015 = 53{,}528\ kg \quad \text{(B.1-127)}$$

The inputs calculated for digestion of this mass of biomass are displayed in table B-11.

Table B-11 Anaerobic digestion inputs following fermentation and distillation process

Input	Value
Mixing	2,068 kWh
Heating	36,918 MJ
Concrete block	116.3 kg
Concrete, normal (slab/roof)	0.028 m^3

For a residual biomass input of 53,528 kg and a methane conversion of 0.25 L CH_4/g VS, the methane yield was calculated to be 8,029 m^3 or 13,056 m^3 biogas. This biogas yield corresponded to an electricity and heat production of 25,612 kWh and 158,473 MJ respectively.

To convert the biogas to electricity and heat, 0.00144 of a 160 kWe co-gen unit with components for electricity and heat, electricity only and heat only were required. The consumption and disposal of 8.6 kg of lubricating oil was also required.

S2 D Biodiesel, bioethanol and biogas production

The inputs to the fermentation and distillation process are based on the mass of residual biomass following lipid extraction. The mass was calculated simply by subtracting the mass of oil extracted. Assuming a 20% recovery of oil the mass of biomass entering the fermentation process was calculated as:

$$M_{fermentation} = 67{,}547 \times 0.8 = 54{,}713kg \quad \text{(B.1-128)}$$

Based on a biomass input of 54,713 kg, the model inputs for the fermentation and distillation process were calculated and displayed in table B-12. The bioethanol yield was considered to be unaffected by the lipid extraction and using the 10.3% conversion rate, a bioethanol yield of 6,957 kg was calculated. The upgrading inputs for this mass of bioethanol are displayed in table B-13.

Table B-12 Fermentation and distillation inputs following biodiesel production

Input	Total input value
Electricity	9,836 kWh
Heat	214,144MJ
Ammonium sulphate	190.9 kg
Diammonium phosphate	190.9 kg
Soda powder	712.5 kg
Sulphuric acid	477.1 kg
Ethanol fermentation plant	$9.79\text{-}\times10^{-6}$

Table B-13 Bioethanol upgrading inputs following biodiesel production

Input	Total input value
Electricity	61.5 kWh
Heat	6,989 MJ
Ethanol fermentation plant	3.69×10^{-7}

The mass of biomass entering the anaerobic digester was calculated as the original mass with the oil content subtracted as well as the produced masses of ethanol and CO_2 subtracted. Considering a lipid removal of 20%, an ethanol conversion of 10.3% and a CO_2 to ethanol ratio of 1.015, the mass of biomass was calculated as:

$$M_{digester} = 67{,}547kg - 12{,}834kg - 67{,}546.7\ g \times 0.103 - 67{,}546.7kg \times 0.103 \times 1.015kg = 43{,}357kg \quad \text{(B.1-129)}$$

Considering a biomass mass of 43,357 kg entering the digester, the model input values calculated are displayed in table B-14.

Table B-14 Anaerobic digestion inputs following biodiesel, fermentation and distillation processes

Input	Value
Mixing	1,675 kWh
Heating	29,904 MJ
Concrete block	94.2 kg
Concrete, normal (slab/roof)	0.027 m^3

For a residual biomass input of 43,357 kg and a methane conversion of 0.25 L CH_4/g VS, the methane yield was calculated to be 6,504 m^3 or 10,575 m^3 biogas. This biogas

yield corresponded to an electricity and heat production of 20,746 kWh and 128,363 MJ respectively.

To convert the biogas to electricity and heat, 0.00117 of a 160 kWe co-gen unit with components for electricity and heat, electricity only and heat only were required. The consumption and disposal of 7.0 kg of lubricating oil was also required.

Digestate de-watering and fertiliser production

The digestate from each process stream was assumed to be used as fertiliser following separation of the solid and liquid phase using centrifugation. The centrifuge was modelled on the Alpha Laval OFX40 nozzle centrifuge with an energy consumption of 1 kWh/m^3. The throughput was based on the maximum nozzle flow rate of 43 m^3/h, one centrifuge unit was sufficient for each process stream. The residual biomass flow rates for each process stream were calculated as 530.7 m^3, 519.2 m^3, 655.2 m^3 and 420.6 m^3 for S2 A, S2 B, S2 C and S2 D respectively. The energy consumption values were therefore 531 kWh, 519 kWh, 655 kWh and 421 kWh respectively.

The mass of the centrifuge was assumed to be 12,700 kg, the mass was assumed to be chromium steel with a life span of 20 years. For each process stream the mass of steel was therefore calculated as:

$$M_{steel} = 12{,}700\ kg \times \frac{1}{20\ y \times 365\ d} = 1.7\ kg \qquad \text{(B.1-130)}$$

The value of the digestate as fertiliser was based on the mass of digestate, the N and P content and the mineralisation rates. The masses of digestate for each process stream were calculated to be 54,713 kg, 53,528 kg, 67,547 kg and 43,357 kg for S2 A, S2 B, S2 C and S2 D. The N and P content of the biomass was based on the molecular formula for green algae ($C_{106}H_{263}O_{110}N_{16}P$) [256]. For every gram of dry digestate, it was calculated stoichiometrically that 0.063 g N and 0.0087 g P was produced. The bioavailability of the nitrogen and phosphorous were taken from the study by Warman and Temeer [262] where bioavailability values of 25% and 8% were calculated for N and P based on sewage sludge. The values of N and P produced as fertiliser for each process stream are displayed in Table B-15.

Table B-15 Value of bioavailable N and P for each process stream

Process stream	Bioavailable N (kg)	Bioavailable P (kg)
S2 A	863	87
S2 B	844	85
S2 C	1,066	108
S2 D	684	69

The avoided impacts of the N and P produced were calculated using the data for ammonium sulphate and superphosphate based on the mass of N and P.

Appendix C: A life cycle assessment of macroalgae (seaweed) cultivation and processing for biofuel production

C.1 Biomass production

Values for the biomass production rate for each scenario were obtained from published material largely based on experimental work conducted around the south of Chile. *Gracilaria chilensis* and *Macrocystis pyrifera* naturally have a different growth rate depending upon cultivation method, location, climate and nutrient availability among many other factors. An upper and lower value of productivity for each cultivation scenario was obtained from published studies with a median value providing the value for the base case. Where the obtained values were presented as fresh weight, a moisture content of 85% was assumed [19]. For long-line cultivation, a space of 2 m was assumed between lines corresponding to a rope length of 5 km per hectare.

The values for Scenario 1, the bottom cultivation of *G. chilensis* assumed sub-tidal cultivation between a depth of 0.75 m and 2.5 m, the lower value being recorded at a depth of 0.75 m and the highest value at 2.5 m in the south of Chile [60]. The values for Scenario 2 were obtained from two studies both of which considered the potential of the cultivation to remove nutrients from nearby salmon farms. A lower value (1.68 kg/m/month) was recorded on a line grown adjacent to a salmon farm over the summer months. An upper value of 2.8 kg/m/month was recorded over the spring months in southern Chile [66], similarly influenced by a salmon farm. The upper yield for Scenario 3 was taken from the same study where a *M. pyrifera* yield of 25 kg/m was recorded over a period of 9 months. The lower yield was taken from a study in the north of Chile where a yield of 22 kg/m was recorded after 120 days of off-shore cultivation [62].

The carbon dioxide (CO_2) uptake of each process stream was calculated as the carbon contained within the fertiliser produced. The carbon contained within the fertiliser was calculated based on the elemental carbon content of the species. The carbon content varies between species but a value of 30% was assumed for both species which is a typical value for macroalgae on a dry weight basis [19]. Based on

the stoichiometry of CO_2, for every kg of C produced 3.67 kg of CO_2 was assumed to be taken from the environment.

C.2 Biofuel production

Values for biofuel yields were taken from a variety of published experimental studies, using the same method as used for the productivity, an upper and lower value were obtained with the median providing the base case.

C.2.1 Bioethanol production

The research that has been conducted on the conversion of macro-algae to bioethanol is limited to a few species, mainly brown algae [64, 113-115]. Since ethanol yields from *G. chilensis* are currently not available in the literature, yields from other *Gracilaria* species were used instead. The lower yield considered in the study was obtained from an experimental study that achieved a yield of 3.6 kg ethanol/kg biomass (d.w) from *Gracilaria verrucosa* [117], a higher yield of 7.9 kg ethanol/kg biomass (d.w) was obtained from *Gracilaria salicornia* [116] using acid and enzymatic hydrolysis followed by fermentation with *Escherichia coli* to produce ethanol.

Ethanol production rates from *M. pyrifera* have not been studied, data from alternative brown macroalgae species was therefore substituted. A lower yield of 10.86 kg ethanol/kg biomass (d.w) was obtained from an experimental study where *L. japonica* was fermented using the yeast, *Debaryomyces occidentalis* [222]. A higher value of 13.2 kg ethanol/kg biomass (d.w) was obtained from a study considering the hydrolysis and fermentation of *Laminaria digitata* using laminarinase for hydrolysis and *Pichia angophorae* for fermentation [64].

C.2.2 Biogas production

Biogas yields obtained were provided as litres of CH_4 per gram of volatiles solids (VS) of dried biomass, the volatile solids content for *G. chilensis* and *M. pyrifera* is 59% for both, taken as an average from Habig *et al.* [127] and Roesijadi *et al.* [63], respectively. Values based on a study using *Gracilaria sp.* [127] were used for the upper yield of biogas production where a mesophilic temperature of 32°C and retention time of 58 days produced a yield of 0.23 L CH_4/g VS. A lower yield of 0.18

L CH_4/g VS was obtained from an experimental study using a temperature of 37°C and a retention time of 82 days [126] . The range of the upper and lower value of *M. pyrifera* was greater with a lower yield of 0.20 L CH_4/g VS and an upper yield of 0.41 L CH_4/g VS both produced under mesophilic conditions [214].

C.2.3 Combined production of bioethanol plus biogas

The dry mass of stillage from the fermentation and distillation process was calculated by subtracting the ethanol and CO_2 produced from the initial biomass. CO_2 emitted during the production of bioethanol was estimated to be produced at a ratio of 1.015 to ethanol [219]. The yields of CH_4 from the stillage were assumed to be similar to those from unfermented biomass, although a lower values might be expected.

C.2.4 Fertiliser credit

The digestate remaining after anaerobic digestion and the stillage following fermentation and distillation (where digestion was not used) were assumed to be used as fertiliser. The fertiliser credit was based upon the amount of nitrogen, phosphorous and potassium in the total dry mass of raw macroalgae (See manuscript Table 5-1) and the bioavailability. The bioavailability values were taken from a study which investigated the bioavailability of N, P and K in sewage sludge [262]. The values used were 25%, 8% and 50% for N, P and K respectively. The avoided cumulative energy demand and environmental impacts were then calculated as per kg of N, P and K as ammonium sulphate, single superphosphate and potassium chloride respectively.

C.3 Unit Process Inputs

This section details the inputs to each of the unit processes modelled for each cultivation scenario (S1-S3) and process stream (P1-P3). As the cultivation processes differ between scenarios, these are described separately whereas the processing steps are detailed collectively as they required the same inputs (See manuscript Tables 5-2 and 5-3).

C.3.1 Scenario 1: Bottom planting of G. chilensis

From personal communication with *G. chilensis* farmers, 9 bundles of 200 g (wet weight) of biomass thalli should be planted on each square metre for high productivity of the biomass. The biomass can be planted once and harvested four times before re-planting. Harvesting was assumed to be conducted only once per year. Each hectare therefore requires 18 tonnes of biomass (wet weight) to be recycled and planted every four years corresponding to a recycle rate of 4.5 t/ha/yr. A shed with lighting was the only input assumed to be required for preparation, the inputs of which were calculated to be 0.67 m^2 of agricultural shed and 5.6 kWh of lighting.

C.3.1.1 Preparation of *G.chilensis* thalli

Shed area

20 m^2 of shed area was assumed sufficient for the preparation of 90,000 bundles (200 g/bundle) of biomass for one hectare of cultivation. The shed was assumed to have a life span of 30 years and was not used for any other processes. Therefore, 0.67 m^2 of shed was used as the shed input modelled as an agricultural shed using Ecoinvent (v2.2) [106].

Lighting

A lighting density of 14 W/m^2 was considered necessary based on the ASHRAE 90^2 energy code for the lighting of office space [263]. 10 days were assumed necessary for preparation of one hectare and 8 hours per day of working time. Therefore the energy of the lighting was calculated as:

$$E_{lighting} = 0.014\,\frac{kW}{m^2} \times 20\ m^2 \times 10\ days \times 8\ hours \times 0.25\,\frac{times}{year} = 5.6\ kWh \quad \text{(C-1)}$$

For the cultivation stage, according to the *G. chilensis* farmers, a diver takes four days to plant one hectare (every four years). The diesel consumption for the vessel used each day was understood to be 30 L or 120 L for the planting of one hectare. The consumption each year for planting one hectare was therefore calculated as 30 L (23.7 kg) of diesel. To calculate the impacts of the diesel consumption by the vessel, the impacts of using the same amount of diesel in the operating barge modelled using

data from Ecoinvent [106] were determined. The impacts related to the production of the boat for planting were based on the amortization of a 10 m fishing vessel requiring the production of 0.71 kg of steel and 0.98 kg of aluminium.

C.3.1.2 Cultivation of bottom planted G.chilensis

The material requirements for vessel production were based on values from a study investigating the material inputs to a 10 m fishing vessel [264]. A total steel requirement of 2,260 kg and an aluminium requirement of 3,120 kg were calculated. The material requirements were split at a ratio of 1:8 between the planting and harvesting processes respectively to model the lower usage for planting. Assuming the vessel serviced 20 ha of cultivation area and had a life span of 20 years, the steel consumption was calculated as:

$$M_{steel} = 2260\ kg \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{20}\frac{y}{y} \times \frac{1}{8}\ \frac{planting\ time}{harvesting\ time} = 0.71\ kg \qquad \text{(C-2)}$$

The aluminium consumption was calculated as:

$$M_{alum} = 3120\ kg \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{20}\frac{y}{y} \times \frac{1}{8}\ \frac{planting\ time}{harvesting\ time} = 0.98\ kg \qquad \text{(C-3)}$$

The harvesting of the biomass was assumed to be carried out by a fisherman and diver using the same fishing vessel. According to the farmers, the harvesting of one hectare takes three days consuming 90 L of diesel. A total diesel consumption of 90 L (71 kg) was therefore considered as the fuel input, the impacts of which were modelled as for the cultivation. The vessel material amortization was calculated as 4.94 and 6.83 kg/ha of steel and aluminium respectively.

C.3.1.3 Harvesting of bottom planted G. chilensis

The vessel production requirements were calculated as in used previously.

Steel consumption was calculated as:

$$M_{steel} = 2260\ kg \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{20}\frac{y}{y} \times \frac{7}{8}\ \frac{planting\ time}{harvesting\ time} = 4.94\ kg \qquad \text{(C-4)}$$

Aluminium consumption was calculated as:

$$M_{alum} = 3120\ kg \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{20}\frac{y}{y} \times \frac{7}{8}\ \frac{planting\ time}{harvesting\ time} = 6.83\ kg \qquad \text{(C-5)}$$

C.3.2 Scenario 2: Long-line cultivation of G. chilensis

The tying of thalli to rope for long-line cultivation of *G. chilensis* is modelled on the study by Abreu *et al.* [65] where 50 g (wet weight) bundles of biomass were attached at 20 cm intervals over the rope length. For each hectare, 1.25 tonnes of biomass was therefore required to be recycled and tied, this amount was subtracted from the total yield of biomass harvested. To allow the tying of thalli an shed input area of 1.33 m^2 requiring 22.4 kWh of lighting was input to the model, the mass of rope was calculated as 111.9 kg of polyamide.

C.3.2.1 Preparation of G. chilensis thalli for rope tying

Shed area

40 m^2 of shed area was considered necessary for the preparation of ropes sufficient for each hectare of cultivation area. The shed life span was 30 years, the area modelled was therefore 1.33 m^3.

Lighting

The same lighting density as was used in for scenario 1 preparation was used but it was assumed that 5 days would be sufficient to seed the lines for one hectare. The energy was therefore calculated as:

$$E_{lighting} = 0.014\frac{kW}{m^2} \times 40\ m^2 \times 5\ days \times 8\ hours = 22.4\ kWh \quad \text{(C-6)}$$

Rope

The rope that the thalli was tied to was assumed to be of 5 mm diameter and made from polyamide. Using data obtained from Lanex a.s. [265], the density of polyamide rope is 1,140 kg/m^3. The mass input of polyamide rope was therefore calculated as:

$$M_{rope} = 5000\ m \times \pi \times 0.0025^2\ m^2\ \times 1140\ \frac{kg}{m^3} = 111.9\ kg \qquad \text{(C-7)}$$

Once tied, the ropes were required to be deployed offshore as depicted in figure 2-2 of the manuscript. Structural lines with a diameter of 20 mm perpendicular to the culture lines were assumed at both ends and one at the mid-point, requiring 300 m total length corresponding to an input of 21.5 kg of polyamide. The position of the lines was maintained by using a series of concrete blocks, chains and buoys, corresponding to input values of 952 kg concrete, 38.4 kg steel and 52.5 kg polyethylene respectively. The ropes and buoys were assumed to be deployed using a barge, this was modelled using data from Ecoinvent [106] for the operation of a barge consuming 9.39×10^{-3} kg diesel/t.km. A diesel consumption of 0.045 kg was calculated. The impact of the barge production was included based on a 30 year life span covering 100 ha of cultivation area and split equally between the cultivation and harvesting process. Observation of the culture was assumed to take place once per month over the six month growing period which consumed 0.68 kg diesel and 0.36 kg aluminium to model.

C.3.2.2 Long-line cultivation of G. chilensis

The culture ropes were assumed to be attached to stronger 20 mm polyamide ropes, which were placed at each end and the mid-point requiring a total of 300 m of rope. The rope mass was calculated using a density of 1,140 kg/m^3 obtained from Lanex a.s. [265], the ropes were assumed to have a life span of 5 years. The mass was calculated as:

$$M_{rope} = 300\ m \times \pi \times 0.01^2\ m^2\ \times 1140\ \frac{kg}{m^3} = 21.5\ kg \qquad \text{(C-8)}$$

For anchorage eight concrete blocks of 1 m^3 volume were considered adequate for one hectare. Concrete blocks were assumed to have a life span of 20 years and a density of 2,380 kg/m^3. The mass of concrete was calculated as:

$$M_{concrete} = 8 \times 1\ m^3\ \times 2380 \frac{kg}{m^3} \times \frac{1}{20} \frac{y}{y} = 952\ kg \quad \text{(C-9)}$$

Steel chains attached the structural ropes to the concrete blocks, the blocks were assumed to be at a depth of 20 m therefore requiring 160 m of chain. The mass of chain was considered to be 4.8 kg/m [266] and was assumed to be manufactured from chromium steel with a life span of 20 years. The mass of chain was calculated as:

$$M_{steel} = 160\ m \times 4.8 \frac{kg}{m} \times \frac{1}{20} \frac{y}{y} = 38.4\ kg \quad \text{(C-10)}$$

Buoys were placed at 20 m intervals on each culture line, requiring a total of 250 per hectare. The buoys used were assumed to be the model type "A2 (20"x16")" weighing 2.1kg [267]. The buoys were modelled using polyethylene as the material of manufacture and were considered to have a life span of 10 years. The total mass of polyethylene was calculated as:

$$M_{PE} = 250 \times 2.1\ kg \times \frac{1}{10} \frac{y}{y} = 52.5\ kg \quad \text{(C-11)}$$

The distance from the shore to the site was assumed to be 5 km and deploying the ropes required another 5 km. The total mass of rope and buoys was 0.637 t. The diesel consumption of the barge was calculated as:

$$M_{diesel} = 9.39 \times 10^{-3} \frac{kg}{t.km} \times 0.637\ t \times (10\ km + 5\ km) \times 0.5 = 0.045\ kg \quad \text{(C-12)}$$

The cumulative energy demand and environmental impacts of the barge production were included. The barge was assumed to service 100 hectares of cultivation area and have a life span of 30 years. The impacts of production were obtained from the Ecoinvent (v2.2) database [106] and where divided equally between the cultivation and the harvesting processes.

Observation

Observation of the long-line culture was assumed to be conducted using a 25 horsepower fishing vessel modelled on the Alumacraft Escape 145 Tiller [268]. The technical specifications suggest a fuel consumption of 10.22 mpg (4.34 km/l) for a velocity of 18.4 mph. Each hectare is visited once a month over a 6 month growing period. For each trip 50 hectares are visited. The fuel consumption to the site was therefore calculated as:

$$M_{diesel} = 10\ km \times \frac{1}{4.34}\frac{l}{km} \times \frac{1}{50}\frac{ha}{ha} \times 6\ \frac{times}{year} \times 0.832\ \frac{kg}{l} = 0.23\ kg\ diesel \quad \text{(C-13)}$$

The fuel consumption on site was calculated by assuming the vessel travels 500 m around each hectare at 2.6 mph. According to technical specifications the fuel consumption at 2.6 mph is 13 mpg (5.53 km/l). The fuel consumption was therefore calculated as:

$$M_{diesel} = 0.5\ km \times \frac{1}{5.53}\frac{l}{km} \times 6\frac{times}{year} \times 0.832\frac{kg}{l} = 0.45\ kg\ diesel \quad \text{(C-14)}$$

The materials consumption of the vessel was based on the vessel mass specified in the technical data. The total mass was 1,567 lb (711 kg) which was assumed to be aluminium. The total mass input was calculated as:

$$M_{alum} = 711\ kg \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{20}\frac{y}{y} = 0.36\ kg\ aluminium \quad \text{(C-15)}$$

Harvesting

Harvesting was modelled using the operation of a barge from Ecoinvent [106] which consumes 9.39 $\times 10^{-3}$ kg/t.km. The mass of biomass, ropes and buoys was calculated as 68.2 t. The diesel consumption in the barge was therefore calculated as:

$$M_{diesel} = 9.39 \times 10^{-3}\frac{kg}{t.km} \times 68.2\ t \times (10\ km + 5\ km) \times 0.5 = 4.78\ kg \quad \text{(C-16)}$$

The impacts of the barge production were divided by the 100 ha cultivation area, a life span of 30 years and divide equally between the cultivation and harvesting processes.

Harvesting was assumed to be conducted using the same barge as used for deployment. The distance was the same at 5 km to the cultivation site and 5 km on-site. The biomass yield combined with the mass of ropes and buoys was 68.2 t, corresponding to an input value of 509 t.km or 4.78 kg of diesel consumed by the barge. Half of the barge impacts were allocated to the harvesting process.

C.3.3 Scenario 3: Long-line cultivation of M. pyrifera

For the production of *M. pyrifera*, a hatchery process was necessary to begin the cultivation of the biomass by inoculating ropes and developing the spores. For spore inoculation, the process begins with stimulating fertile thalli to release spores into a tank containing seawater and rope for cultivation [217]. This process can be completed in 48 hours and was not included in the model due to its relative insignificance. Prior to transplantation offshore, the spores needed to develop. This step was modelled on the study by Macchiavello *et al.* [62] in which the authors developed spores of *M. pyrifera* under laboratory conditions. The inputs to this study were extrapolated for a larger growing area of one hectare. The inputs calculated were 50.4 kg of polyamide rope, 0.25 kg ammonium nitrate, as N, 0.47 kg Sodium phosphate, 971.7 kWh of electricity for water pumping, water treatment, aeration and lighting and 0.67 m^3 of agricultural shed.

C.3.3.1 M. pyrifera hatchery

The hatchery process was modelled on the method used by Macchiavello *et al.* [62]. In their study, polypropylene rope with a diameter of 3 mm was used for attachment of the spores. A 20 L aquarium was used for spools containing 30 metres of rope. The temperature was maintained at 15°C and two lamps of 40 W were used on a 12:12 hour light period. Aeration was constant for the culture period of 60 days. These values were used but adapted where necessary and extrapolated for a cultivation area of one hectare.

Materials and lighting

This study assumed a two metre distance between rope lines. Therefore, 50 lines of 100 metre length were assumed for one hectare of area requiring 5000m of rope. As the rope needs to be wound around support ropes, a length of 6,250m of rope was

used. A density of polyamide rope of 1140 kg/m^3 was obtained from Lanex a.s. [265]. The mass or rope was calculated as:

$$M_{rope} = 6250\ m \times \pi \times 0.0015^2\ m^2 \times 1140\ \frac{kg}{m^3} = 50.4\ kg \qquad \text{(C-17)}$$

A tank volume of 4.17 m^3 was assumed necessary to accommodate the rope required for one hectare during spore inoculation. The study by Macchiavello *et al.* [62] used two 40 W lamps for 20 L of aquarium, for this study eight 40 W lamps were assumed to be sufficient per cubic metre of tank volume on a 12:12 hour cycle. The energy consumption was therefore calculated as:

$$E = 8\frac{lamps}{m^3} \times 4.17\ m^3 \times 40\ W \times 12\ h \times 60\ days = 960\ kWh \qquad \text{(C-18)}$$

Nutrient provision

The medium for spore growth was considered to be Von stosch medium which requires 42.5 mg/L of $NaNO_3$ and 10.75 mg/L of Na_2HPO_4 [269]. The concentrations of the other nutrients were considered to be negligible and were ignored. The masses of nutrients required were calculated considering the total water replacement once a week over 60 days which equalled 35.7 m^3. The total N requirement was calculated stoichiometrically as:

$$N = 42.5\frac{g}{m^3} \times 0.165\ \frac{g\ N}{g\ NaNO_3} \times 35.7 m^3 = 0.25\ kg \qquad \text{(C-19)}$$

The total mass of Na_2HPO_4 was calculated as:

$$Na_2HPO_4 = 10.75\frac{g}{m^3} \times 35.7 m^3 = 0.38\ kg \qquad \text{(C-20)}$$

As data for the CED and environmental impacts of sodium nitrate were not available, the environmental impacts and CED of 0.25 kg (nitrogen) of ammonium nitrate were modelled instead using data from Ecoinvent (v2.2) [106]. The total Na_2HPO_4 mass required was modelled as sodium phosphate.

Water pumping

The water for replacing the tank volume was assumed to be pumped from the coast near to the hatchery. The power consumption for water pumping was calculated from the equation for a pump taken from Chadwick *et al.* [250]:

$$P = 6.89 \times 10^{-6} \frac{m^3}{s} \times 1025 \frac{kg}{m^3} \times 9.81 \frac{m}{s^2} \times 5\ m \times \frac{1}{0.8} = 0.43\ W \quad \text{(C-21)}$$

The water flow is the total volume of water for tank volume replacement over the 60 days which was 35.7 m^3. The total dynamic head was assumed to be 5m as the hatchery was considered to be located beside the coast and frictional head was neglected. The energy consumption over the culture period was therefore 0.62 kWh.

Air pumping

24 hour aeration of the culture tanks was assumed to be conducted using a 0.75 hp sweetwater centrifugal pump [270] which was considered sufficient to pump air for each tank developing spores for the total area (100 ha). A horsepower to watt conversion of 745.7 was used which corresponds to a power rating of 0.56 kW or 5.6 W/ha. The pump was operated on a 24 hour basis over the total culture period of 60 days, therefore the energy consumption was calculated as:

$$E_{air} = 0.75\ hp \times 745.7 \frac{w}{hp} \times 24\ h \times 60\ d \times \frac{1}{100} \frac{ha}{ha} = 8.05\ kWh \quad \text{(C-22)}$$

Water treatment

The water treatment was modelled on a unit produced by DrydenAqua Ltd with a sub-sand filter and AFM active filter media in a pressure sand filter followed by a 1 micron pleated polyester filter elements to give 1 micron absolute filtration. According to personal correspondence with the manufacturers, the unit has a throughput of 20 m^3/hr and a power rating of approximately 1.5 kW.

The energy consumption was calculated as:

$$E_{WT} = 1.5\ kW \times \frac{35.7}{20} \frac{m^3}{m^3/h} = 2.7\ kWh \quad \text{(C-23)}$$

The water was also assumed to also be treated using UV disinfection based on information from Infralight Technology [271]. The unit SF940 has a design flow of 75 L/min and a power rating of 40 W. The energy consumption was calculated as:

$$E_{UV} = 0.04\ kW \times \frac{35.7}{4.5} \frac{m^3}{m^3/h} = 0.32\ kWh \quad \text{(C-24)}$$

Building

The assumed building area required for each hectare of cultivation area was 20 m^2 of shed and the shed was considered to have a life span of 30 years. The area of shed input to the model was therefore 0.67 m^2. The temperature of the building was assumed not to require control however depending upon the location of the hatchery and the time of year this could potentially increase the energy consumption.

Once the spores were developed after 60 days, the lines were assumed to be wound around stronger 10 mm polyamide ropes requiring 110 kg of polyamide rope. The offshore cultivation method of *M. pyrifera* was the same as scenario 2 as depicted in figure 2-2 (main manuscript) using the same set-up of concrete blocks, buoys and steel chains. The total mass of ropes and buoys was calculated as 1,023 kg which was assumed to be transported by barge consuming 0.072 kg of diesel. The impact of the barge fabrication was included using the same method as scenario 2. Observation was conducted once a month, in this case over a nine month growth period consuming 1.02 kg diesel and 0.36 kg aluminium. The concrete, steel and polyethylene material inputs were the same as for scenario 2.

Harvesting was modelled using the barge that was used for deployment. The mass of biomass produced as well as the ropes and buoys was calculated as 118.5 t, corresponding to a diesel consumption of 8.35 kg. The impacts of the barge production were divided equally between the harvesting and cultivation processes.

C.3.3.2 Cultivation of M. pyrifera

Support ropes

The culture ropes were assumed to be wound around stronger 10 mm polyamide ropes. A total length of 5,000 m of support rope was necessary. 3 lengths of 100 m of additional structural rope with a diameter of 20 mm was assumed to be used for attachment of the support ropes and culture ropes. The rope mass was calculated using a density of 1,140 kg/m3 obtained from Lanex a.s. [265], the ropes were assumed to have a life span of 5 years. The mass was calculated as:

$$M_{rope} = (5000\ \text{m} \times \pi \times 0.005^2\ \text{m}^2 + 300\ \text{m} \times \pi \times 0.01^2\ \text{m}^2) \times 1140\ \frac{\text{kg}}{\text{m}^3} \times \frac{1\ \text{y}}{5\ \text{y}} =$$

$$111.0\ \text{kg} \qquad \text{(C.1-25)}$$

The mass of structural ropes, buoys, steel chains and concrete blocks were the same as detailed for the long-line cultivation of *G.chilensis.*

Deployment

$$M_{diesel} = 9.39 \times 10^{-3} \frac{kg}{t.km} \times 1.023\ t \times (10\ km + 5\ km) \times 0.5 = 0.072kg$$

(C-25)

Observation

Using the same vessel and assumptions as for long-line cultivation of *G.Chilensis*, the diesel consumption was calculated as:

$$M_{diesel} = 10km \times \frac{1}{4.34} \frac{l}{km} \times \frac{1}{50} \frac{ha}{ha} \times 9 \frac{times}{year} \times 0.832 \frac{kg}{l} = 0.34\ kg\ diesel \quad \text{(C-26)}$$

The fuel consumption on site was calculated by assuming the vessel travels 500 m around each hectare at 2.6 mph. According to technical specifications the fuel consumption at 2.6 mph is 13 mpg (5.53 km/l). The fuel consumption was therefore calculated as:

$$M_{diesel} = 0.5km \times \frac{1}{5.53} \frac{l}{km} \times 9 \frac{times}{year} 0.832 \frac{kg}{l} = 0.68\ kg\ diesel \quad \text{(C-27)}$$

The vessel production was assumed to be the same as that used for the long-line cultivation of *G.Chilensis* requiring 0.36 kg aluminium.

Harvesting

The total mass of biomass, ropes and buoys to be harvested was calculated to be 118.5 t, based on the diesel consumption of the barge the mass of diesel consumed was calculated as:

$$M_{diesel} = 9.39 \times 10^{-3} \frac{kg}{t.km} \times 118.5\ t \times (10\ km + 5\ km) \times 0.5 = 8.35kg$$

(C-28)

Pre-processing

The pre-processing method was the same for each scenario. Prior to processing, the biomass was transported from the boat landing point to the processing facilities and then ground. The facilities were assumed to be 100 m from the point where the boat landing point was located. The method of transport was a 100 m long conveyor belt with a power rating of 0.68 kW, the electricity consumption depended upon the yield of biomass being transported. The conveyor belt transported the biomass to a wet grinding attritor, with a power rating of 93.2 kW. The calculations for energy consumption depended upon the biomass throughput and material allocation depended upon cultivation area.

C.3.4 Conveyor belt design

A conveyor belt was assumed to transport the harvested biomass from the point where the boats land to the processing units. This distance was considered to be 100 m. The conveyor belt was designed using data from Rulli Rulmeca [272]. A belt with width 500 mm was chosen and angle of surcharge 5° on a flat roller set. The fixed coefficient of resistance was 2.1, the passive coefficient of resistance, 1, the coefficient of friction for internal rotating parts, 0.016, the belt weight per linear meter, 3.45 kg/m, the weight of lower rotating parts, 1.2 kg/m, the weight of upper rotating parts, 3.09 kg/m, weight of conveyed material, 3.5 kg/m and the height change, 3m.

The tangential force was calculated as:

$$F_u = \left(100m \times 2.1 \times 0.016 \times \left(2 \times 3.45\,\frac{kg}{m} + 3.5\,\frac{kg}{m} + 1.2\,\frac{kg}{m} + 3.09\,\frac{kg}{m}\right) + \left(3.5\,\frac{kg}{m} \times 3m\right)\right) \times 0.981 = 58.8\ \text{N} \quad \text{(C-29)}$$

The belt velocity was assumed to be 1 m/s and an efficiency of reduction gear of 0.86. The belt driving power was calculated as:

$$P = \frac{58.8\ N \times 1\frac{m}{s}}{100 \times 0.86} = 0.68\ kW \quad \text{(C-30)}$$

The energy consumption of the conveyor belt was calculated by multiplying the time taken to transport the biomass by the driving power of the conveyor belt. The time taken was calculated by dividing the volume of biomass harvested by the load volume which for the size of the belt was 12.6 m^3/h.

The material consumption was calculated as the mass of steel of the upper rotating parts (3.09 kg/m) and the lower rotating parts (1.2 kg/m) and the mass of rubber (3.45 kg/m). The steel parts were assumed to have a life span of 10 years and the rubber belt a life span of 5 years.

C.3.4.1 Scenario 1 (Bottom cultivated *G.chilensis*)

Energy consumption:

$$E = 0.68\ kW \times \frac{1}{12.5}\ \frac{h}{m^3} \times 101.5\ m^3 = 5.5\ kWh \quad \text{(C-31)}$$

Material consumption:

$$M_{steel} = \left(3.09\frac{kg}{m} + 1.2\frac{kg}{m}\right) \times 100\ m \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 2.16\ kg \quad \text{(C-32)}$$

$$M_{rubber} = \left(3.45\frac{kg}{m}\right) \times 100\ m \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{5}\frac{y}{y} = 3.45\ kg \quad \text{(C-33)}$$

C.3.4.2 Scenario 2 (Long line cultivated *G.chilensis*)

Energy consumption:

$$E = 0.68\ kW \times \frac{1}{12.5}\ \frac{h}{m^3} \times 66.0\ m^3 = 3.58\ kWh \quad \text{(C-34)}$$

Material consumption:

$$M_{steel} = \left(3.09\frac{kg}{m} + 1.2\frac{kg}{m}\right) \times 100\ m \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 0.43\ kg \quad \text{(C-35)}$$

$$M_{rubber} = \left(3.45\frac{kg}{m}\right) \times 100\ m \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{5}\frac{y}{y} = 0.69\ kg \quad \text{(C-36)}$$

C.3.4.3 Scenario 3 (Long line cultivated *M.pyrifera*)

Energy consumption:

$$E = 0.68\ kW \times \frac{1}{12.5}\ \frac{h}{m^3} \times 117.5\ m^3 = 6.38\ kWh \quad \text{(C-37)}$$

Material consumption:

$$M_{steel} = \left(3.09\frac{kg}{m} + 1.2\frac{kg}{m}\right) \times 100\ m \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 0.43\ kg \quad \text{(C-38)}$$

$$M_{rubber} = \left(3.45\frac{kg}{m}\right) \times 100\ m \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{5}\frac{y}{y} = 0.69\ kg \quad \text{(C-39)}$$

C.3.5 Grinding attritor

The grinding attritor is based on the Q-100 unit produced by Union Process [259]. The unit has a throughput of 130 gallons per minute (32.7 t/h), an average power requirement of 125 horsepower (93.2 kW) and a weight of 9,900 pounds (4,491 kg). The attritor was assumed to be produced from chromium steel and have a life span of 10 years.

C.3.5.1 Scenario 1 (Bottom cultivated *G.chilensis*)

Energy consumption:

$$E = 93.2\ kW\ \times \frac{1}{32.7}\frac{h}{t} \times 101.5\ t = 289.3\ kWh \quad \text{(C-40)}$$

Material consumption:

$$M_{steel} = 4491\ kg \times \frac{1}{20}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 22.5\ kg \quad \text{(C-41)}$$

C.3.5.2 Scenario 2 (Long line cultivated *G.chilensis*)

Energy consumption:

$$E = 93.2\ kW\ \times \frac{1}{32.7}\frac{h}{t} \times 66.0\ t = 188.0\ kWh \quad \text{(C-42)}$$

Material consumption:

$$M_{steel} = 4491\ kg \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 4.49\ kg \quad \text{(C-43)}$$

C.3.5.3 Scenario 3 (Long line cultivated *M.pyrifera*)

Energy consumption:

$$E = 93.2\ kW\ \times \frac{1}{32.7}\frac{h}{t} \times 117.5\ t = 334.9\ kWh \quad \text{(C-44)}$$

Material consumption:

$$M_{steel} = 4491\ kg \times \frac{1}{100}\frac{ha}{ha} \times \frac{1}{10}\frac{y}{y} = 4.49\ kg \quad \text{(C-45)}$$

C.3.5 Processing

Two processing methods were modelled in the LCA study, fermentation/distillation to bioethanol and anaerobic digestion to biogas. No studies have been conducted investigating the energy use or environmental impacts of energy recovery from macro-algal biomass on a large scale, therefore for fermentation/distillation the model relied upon Ecoinvent data [219] for first generation biomass and the anaerobic digestion of the biomass was modelled using data for sludge digestion [200].

Fermentation and distillation are the processing methods of converting organic material to ethanol through biological and thermal processes. Bioethanol fuel requires a purity of 99.7% and can be mixed with fossil fuels up to a proportion of 85% in specialised engines [236]. The cumulative energy demand and impacts were calculated using data from the Ecoinvent database [106] for the electricity, heat and material consumption required to produce 99.7% bioethanol from corn feedstock. The material and energy inputs were adjusted to the macroalgae as a feedstock based on the carbon content ratio of the corn and the algae. As the moisture content of the macroalgae was much higher, the contribution of plant use was based on the fresh mass of biomass into the fermentation/distillation plant.

C.3.5.1 Fermentation and distillation of biomass

Fermentation and distillation was based on the production of bioethanol from corn modelled by Ecoinvent [219]. The inputs to the fermentation and distillation process used by Ecoinvent for producing 1kg of bioethanol (95%) are displayed in Table C-1. The values of electricity and heat include the input for pretreatment, saccharification, fermentation and distillation but not for stillage treatment.

Table C-1. Inputs to the production of 1 kg of bioethanol (95%) from corn

Input	Unit	Value
Corn	kg	3.226
Electricity	kWh	0.134
Heat	MJ	3.631
Ammonium sulphate	kg, as N	9.655×10^{-3}
Diammonium phosphate	kg, as N	9.655×10^{-3}
Soda powder	kg	3.607×10^{-2}
Sulphuric acid	kg	2.404×10^{-2}
Ethanol fermentation plant		2.525×10^{-10}

The inputs to the study were modified by adjusting the values to the equivalent carbon content of the macro-algae. According to Ecoinvent [219], the carbon content of the corn grains are 0.375 kg per kg of fresh mass (37.5%). The carbon content of both macroalgae species was assumed to be 30% of the dry mass or 4.5% of the fresh mass, therefore each input was multiplied by:

$$Conversion\ factor = \frac{0.045}{0.375 \times 3.226\ \frac{1}{kg}} \times mass\ of fresh\ biomass\ kg \quad \text{(C-46)}$$

The fraction of the ethanol fermentation plant was calculated by considering the mass of biomass into the plant. The contribution of the plant for 3.226 kg of corn was multiplied by the mass of macroalgae biomass (w.w) and divided by 3.226.

Upgrading bioethanol to 99.7%

The process of upgrading the bioethanol from 95% to 99.7% was modelled on information from Ecoinvent [219], the inputs are displayed in Table C-2 for 1 kg of bioethanol 99.7%. The total input for upgrading was calculated by multiplying the input values by the total ethanol produced in each scenario, the values are displayed in Table C-3.

Table C-2. Inputs required for upgrading bioethanol from 95% to 99.7%

	Unit	Value
Corn	kg	3.226
Electricity	kWh	0.134
Heat	MJ	3.631
Ammonium sulphate	kg, as N	9.655×10^{-3}
Diammonium phosphate	kg, as N	9.655×10^{-3}
Soda powder	kg	3.607×10^{-2}
Sulphuric acid	kg	2.404×10^{-2}
Ethanol fermentation plant		2.525×10^{-10}

Table C-3. Inputs for each scenario for the fermentation and distillation process

Input	Unit	Scenario 1	Scenario 2	Scenario 3
Mass of fresh biomass	t	101.5	66.0	117.5
Electricity	kWh	507.2	329.6	587.2
Heat	MJ	13,707.2	8,906.3	15,868.0
Ammonium sulphate	kg, as N	36.5	23.7	42.2
Diammonium phosphate	kg, as N	36.5	23.7	42.2
Soda powder	kg	136.2	88.5	157.6
Sulphuric acid	kg	90.8	59.0	105.1
Ethanol fermentation plant		7.946×10^{-6}	5.163×10^{-6}	9.198×10^{-6}
Electricity (upgrading)	kWh	7.9	5.1	18.8
Heat (upgrading)	MJ	894.8	581.4	2,131.5
Ethanol fermentation plant (upgrading)		4.72×10^{-8}	3.07×10^{-8}	1.12×10^{-7}

Alternative data tested for fermentation and distillation

Input data for fermentation and distillation was also tested using data that was used in an alternative LCA study [215] which based data on an experimental study [39]. The inputs per MJ of bioethanol are displayed in Table C-4.

Table C-4. Alternative inputs for fermentation/distillation of microalgae per MJ of bioethanol

Process	Energy consumption ($MJ/MJ_{ETOH)}$
Fermentation	0.056
Vapour compression steam stripping (Heat)	0.161
Molecular sieve (Heat)	0.056
Vapour compression steam stripping (Electricity)	0.051
Vapour compression distillation (Electricity)	0.067

The energy consumption values were multiplied by the lower heating value of the bioethanol produced.

The study from which the data was taken [215] does not include the fermentation/distillation infrastructure however for consistency this study included the ethanol fermentation plant modelled using data from Ecoinvent [219]. The contribution of the plant was calculated using the same methodology based on the ratio of carbon content of the macroalgae to corn.

Anaerobic digestion is the process which facilitates the production of biogas through the bacterial transformation of biomass. The process requires an anaerobic tank to contain the biomass and a method of sludge heating and mixing. The temperature of the tank depends upon the desired conditions, this study considered the use of mesophilic conditions, a temperature of 37°C was assumed.

The input data related to electricity, heat and material consumption was calculated using information and data from the design of anaerobic digestion systems for digestion of sludge in wastewater treatment plants [200]. The design was based on a simple mechanical mixing tank constructed of concrete which was assumed to operate at 37°C with a retention time of 38 days.

C.3.5.2 Anaerobic digestion of biomass

Anaerobic digestion of the macroalgae was modelled based on data and information for sludge digestion [200]. The mixing power of the digester is dependent upon the volume of the digester. According to Metcalf and Eddy [200], the power requirement for mixing 1 m^3 of digester volume is between 0.005 and 0.008 kW. An average value of 0.0065 kW/m^3 was used in this study. The digester volume necessary to accommodate one hectare's yield of biomass was calculated by multiplying the influent volume of biomass by the retention time (38 days). The density of the algal sludge was taken to be 1,000 kg/m^3. The mixing energy was calculated as:

$$E_{mixing} = 0.0065 \frac{kW}{m^3} \times Biomass\ volume\ m^3 \times 38\ d \times 24\ h \qquad \text{(C-47)}$$

The anaerobic digestion was assumed to operate at a temperature of 37°C, the biomass sludge entering the digester therefore had to be heated. The energy required to heat the sludge was calculated based on the mass of incoming sludge and the temperature difference between the digester and the sludge. The heat capacity was assumed to be the same as water (4.2 J/kg.°C) and the environmental temperature was assumed to be 20°C. The calculation was therefore:

$$E_{heating} = influent\ mass\ kg\ \times (37℃ - 20℃) \times 4200 \frac{J}{kg.℃} \times \frac{1}{3600} \qquad \text{(C-48)}$$

The heat required to account for heating loss in the digester was calculated based on heat transfer coefficients from Metcalf and Eddy [200], for plain concrete walls above ground with insulation (0.7 W/m^2.°C), a plain concrete floor with dry earth (1.7 W/m^2.°C) and a 100 mm thick, covered and insulated concrete fixed cover (1.4 W/m^2.°C). The areas of the floor, cover and walls were determined by the volume of biomass sludge. For each hectare the digester was assumed to be cylindrical with a diameter of 6m and a corresponding floor and ceiling area of 28.3 m^2. The wall

areas were calculated by dividing the volume of influent biomass by the floor area. The heat energy required for the heat loss was therefore calculated as:

$$E_{h\ loss} = (A_{wall}\ m^2 \times 0.7 \frac{W}{m^2.℃} + A_{base}\ m^2 \times (1.7 + 1.4)\ \frac{W}{m^2.℃}) \times (37℃ - 20℃) \times 24\ h \times 38\ d \qquad \text{(C-49)}$$

The impacts related to the infrastructure for anaerobic digestion were calculated based on the area and depth of the walls, roof and floors. The walls and floors were assumed to be 300 mm thick concrete and the cover from 100 mm concrete. The impacts were calculated using data from Ecoinvent (v2.2) [106] for concrete with a density of 2,380 kg/m^3 and were divided by the life span of the digester (30 years). The digester inputs for process stream P2 and P3 are displayed in Tables C-5 and C-6 respectively.

Table C-5. Anaerobic digestion process inputs for each scenario for process steam, P2

Input	Unit	Scenario 1	Scenario 2	Scenario 3
Mass of fresh biomass	T	101.5	66.0	117.5
Electricity (mixing)	kWh	601.7	391.0	696.5
Heat (heating)	MJ	14,783.0	11,318.8	16,342.2
Concrete	kg	2,776.6	2,184.4	3,043.2

Table C-6. Anaerobic digestion process inputs for each scenario for process stream, P3

Input	Unit	Scenario 1	Scenario 2	Scenario 3
Mass of fresh biomass	T	89.5	58.2	89.0
Electricity (mixing)	kWh	530.8	344.9	527.6
Heat (heating)	MJ	13,617.1	10,561.2	13,564.7
Concrete	kg	2,577.3	2,054.9	2,568.4

C.4 Modelling of the Chilean national grid

The electricity supply from the national grid in Chile was modelled using information from a study produced for the Global Energy Network Institute [273]. The contributions of different sources to the national grid are detailed in table C-7 below alongside the data that was used for the model. All data for modelling of the national grid was taken from Ecoinvent (v2.2) [106].

Table C-7. Contribution of each source of electricity to the Chilean national grid and the source of data for the LCA model

Source	(%)	Data used	Location
Natural gas	36	Electricity, Industrial gas, at power plant	UCTE
Coal	17	Electricity, hard coal, at power plant	US
Diesel (Modelled as oil)	7	Electricity, oil, at power plant	UCTE
Hydroelectricity (reservoir)	27	Electricity, hydropower, at reservoir power plant, non alpine regions	RER
Hydroelectricity (run-of-river)	11	Electricity, hydropower, at run-of-river power plant	RER
Wood	2	Electricity, at cogen 6400kWth, wood, allocation energy	CH
Wind	0.1	Electricity, at wind power plant	RER

(Note: UCTE - Union for the coordination of transmission of electricity (Europe); RER - Europe; CH – Switzerland)